NAVAL MEDICAL RESEARCH UNIT– DAYTON

Health Risk Assessment of Women in Submarines (Phase III): Two Generation Developmental and Reproductive Safety Evaluation of Major Submarine Atmosphere Components (CO, CO_2, and O_2) in Rats (Rattus Norvegicus)

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Abstract

This study evaluated general, reproductive, and developmental effects on male and female rats exposed to mixed atmospheres of three critical submarine air components (CO, CO_2, and O_2) at concentrations approximating the existing submarine standards for continuous exposure limits (CELs) and emergency exposure limits (24-hour EEL and 1-hour EEL). This report describes a 90-day, two-generation evaluation of the general health and reproductive effects in male and female rats exposed to atmospheres representing the Navy's current limits. This study also evaluated the development and reproductive ability of first generation offspring exposed *in utero* to gestation day (GD) 19, and the development of the unexposed second generation offspring. Four groups of 32 male and 32 female rats were exposed *via* whole body inhalation to clean air (0.4 ppm CO; 0.13% CO_2; 20.6% O_2), a low-dose gas mixture (5.0 ppm CO; 0.41% CO_2; 17.1% O_2), a mid-dose gas mixture (13.9 ppm CO; 1.20% CO_2; 16.1% O_2) and a high-dose gas mixture (89.9 ppm CO; 2.5% CO_2; 15.0% O_2) for 23 hours per day for 70 days, followed by a 14-day mating period that was also under exposure. Impregnated dams continued exposure to GD 19. Male and female rats were exposed for 90 to105 days. No adverse reproductive effects were identified in either the exposed parents or first generation offspring during mating, gestation or parturition. There were no adverse changes to the estrous cycle, or in reproductive hormone concentrations, due to the exposures. The only exposure-related effects were reduced weight gains of marginal biological significance and a normal adaptive up-regulation of erythropoiesis, both effects being most notable in male rats from the high-dose group. There were no adverse, dose-related health effects identified in either the exposed parents or offspring based on clinical data (hematology; serum chemistry) or on physiological data (gross pathology; histopathology; organ weights). Additionally, neurobehavioral tests of emotionality, exploratory behavior, motor activity, and cognitive functions (learning and memory) identified no apparent developmental deficits.

Keywords: Inhalation, carbon monoxide, carbon dioxide, developmental toxicity, fertility, gestation, hypoxia, reproductive toxicity, submarine atmosphere

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Introduction

Submarine atmospheres present a unique and closed occupational environment, with personnel being potentially exposed to low-level concentrations of chemicals and chemical mixtures for 24 hours per day for up to 90 days. Congress has recently passed legislation that will allow women to serve aboard submarines; therefore, it is imperative to re-evaluate the current submarine breathing air standards, such as emergency exposure levels (EELs) and continuous exposure levels (CELs), with a special focus upon potential reproductive and developmental effects, as well as sex-specific effects. Based on previous evaluations (National Research Council 2007, 2008, 2009), the atmospheric components of carbon monoxide (CO), carbon dioxide (CO_2), and oxygen (O_2) are considered among the highest health concerns for submarine atmospheres.

Several studies have examined the developmental and reproductive effects of hypoxia, and the inhalation of elevated CO or CO_2 concentrations. Epidemiological evidence attributes several developmental and reproductive effects in humans to hypoxia and CO exposure (Bass et al., 2004; Salam et al., 2005). Published studies in hypoxic animals have indicated decreases in mating rates, sperm production and litter sizes at exposures of 12% O_2 (Cikutovic et al., 2009) and neurobehavioral deficits at exposures of 9.5% O_2 (Chahbourne et al., 2009; Dubrovskaya and Zhravin, 2010). Published findings implicate high CO_2 inhalation exposures with reversible degenerative changes in the testes of rats at 2.5% CO_2 (Vandemark et al., 1972); decreased sperm production in mice at 3.5% CO_2 (Mukherjee and Singh, 1967); decreased fetal viability and increased heart malformations in rats at 6% CO_2 (Haring, 1960); and, neurobehavioral deficits in rats at 7% CO_2 (De la Fuente et al., 2003). In addition, published findings implicate high CO inhalation exposures with decreased fetal weight and viability in mice at 125 ppm (Singh and Scott, 1984), rats at 125 ppm (Prigge and Hochrainer, 1977; Carmines et al., 2007), and rabbits at 90 ppm (Astrup et al., 1972); placental hypertrophy in rats at 100 ppm (Lynch and Bruce, 1989); decreased hematopoiesis in rats at 250 ppm (Prigge and Hochrainer, 1977);

decreased splenic macrophage function in rats at 75 ppm (Giustino et al., 1993); skeletal malformations in mice at 250 ppm (Schwetz et al., 1979); and, neurobehavioral deficits in mice at 125 ppm (Singh, 1986) and rats at 75-150 ppm (Di Giovanni et al., 1995; De Salvia et al, 1995). However, there are no data that assess the combined effects of these three gases as mixtures, nor that assess the adverse health effects of these gases after prolonged, continuous (24 hour per day) exposures.

Assessing the health risk to female crew members in submarines is a complex and controversial issue (Kane and Horn, 2001). This research is being conducted to clarify the potential impacts of these mixed gases on male and female reproductive and developmental health, as well as the overall mission effectiveness of the submarine community. When adequate human data are lacking, the primary alternate method for establishing the health risk from a chemical substance is to perform toxicity studies in animals, and then use the research principles that have been proven to be predictive, robust, and valid for extrapolating the animal results to humans.

The purpose of this study was to evaluate the general, reproductive and developmental toxicity in male and female rats exposed for 90 days *via* whole body inhalation to combinations of the three major submarine atmospheric components (increased CO and CO_2, and decreased O_2).

Experimental Design

The study was performed in three consecutive phases. Phase 1 was a range finding study described in a previous report (NAMRU-D-11-35) and involved a continuous exposure to male and female rats for 14 days to the test atmospheres with toxicity assessments performed on vital organs and reproductive tissues. Phase 2 (NAMRU-D-12-03) described male and female rats exposed for 28 days with neurological and reproductive performance assessed, in addition to general toxicity. Phase 2 offspring were not exposed to the test atmospheres, but were also

assessed for general health conditions and gross malformations. This current report (Phase 3) describes the results of a modified 90-day, two generation, sub-chronic study modeled after U.S. EPA guidelines for assessing "*Reproduction and Fertility Effects*" (OPPTS 870.3800). Male and female rats were exposed to the same test atmospheres that were used during Phases 1 and 2, but for a continuous 90-day period, which included "in-chamber" mating and gestation. Exposed rats were assessed for general toxicity, as well as for neurological and reproductive effects. First generation (F1) offspring were exposed *in utero* up to gestation day (GD) 19, and were evaluated for general toxicity, gross malformations, and neurological and reproductive abilities. Second generation (F2) offspring of randomly selected F1 rats were not exposed to the test atmospheres, but were evaluated for general toxicity, gross malformations and neurological abilities to assess any delayed developmental effects or toxicity.

Materials and Methods

Animal Exposure

The targeted CO, CO_2 and O_2 mixed gas exposure concentrations were selected based upon existing and proposed standard limits promulgated within the *U.S. Navy Technical Manual for Nuclear Powered Submarine Atmosphere Control* (NAVSEA S9510-AB-ATM-010 REV 2). The low-dose group target mixture (5 ppm CO, 0.4% CO_2, 17% O_2) was selected to represent the CEL, which is a composite of the typical chemical concentrations in a submarine atmosphere. The mid-dose group target mixture (14 ppm CO, 1.2% CO_2, 16% O_2) was selected to represent the 24-hour EEL, which is a composite of the maximum permissible chemical concentrations in a submarine atmosphere for a period of 24 hours. The high-dose group target mixture (90 ppm CO, 2.5% CO_2, 15% O_2) was selected to represent the 1-hour EEL, which is a composite of permissible chemical concentrations in a submarine atmosphere for a period of one hour.

Two exposure systems were used during the study. Whole body inhalation chambers provided nearly continuous inhalation exposures to the test animals for at least 90 days to evaluate the developmental and reproductive effects resulting from sub-chronic exposures to the three test atmospheres. However, due to the short biological half-life of carboxyhemoglobin (COHb) in rats of 23 minutes (Anderson et al, 1991), and the excessive time required to draw blood from animals that are individually caged inside of whole body inhalation chambers, a more accessible exposure system was required to determine saturated blood gas levels. Therefore, a nose-only exposure system (NOES) provided inhalation exposures to equivalent groups of animals using atmospheres transferred from the whole body inhalation chambers. Animals were exposed by the NOES for at least 90 minutes before blood samples were collected from each tail vein.

Four groups of animals were exposed to clean air (0.4 ppm CO, 0.13% CO_2, 20.6% O_2), a low-dose gas mixture (5.0 ppm CO, 0.41% CO_2, 17.1% O_2), a mid-dose gas mixture (13.9 ppm CO, 1.20% CO_2, 16.1% O_2) or a high-dose gas mixture (89.9 ppm CO, 2.50% CO_2, 15.0% O_2) for 23 hours per day for 90 to 105 consecutive days (90 days for males and up to 105 day for females to accommodate 19 days of gestation). Each exposure group was stagger-started by three days to minimize disturbance of animals and maximize resources during loading and unloading operations.

Nose-Only Exposure System (Blood Gas Assessment)

Four groups of five male rats and five female rats were exposed via nose-only inhalation to clean air (0.3 ppm CO, 0.13% CO_2, 21.0% O_2), a low-dose gas mixture (5.2 ppm CO, 0.42% CO_2, 17.1% O_2), a mid-dose gas mixture (14.2 ppm CO, 1.20% CO_2, 16.1% O_2) and a high-dose gas mixture (92.6 ppm CO, 2.50% CO_2, 15.0% O_2) for 90-105 minutes.

Animals

A total of 256 CD® IGS rats, 51-54 days-old, were purchased from Charles River Laboratories (Wilmington, MA). The rats were randomly divided into four groups of 32 males and 32 females. The rats were provided husbandry conditions consistent with practices recommended by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), and in compliance with the National Research Council's "Guide for the Care and Use of Laboratory Animals" (ISBN-10:0-309-15400-6). After arrival at the facility, the rats underwent a two week quarantine period in the animal vivarium, which included four days to acclimate to the exposure cage units (cage training). Rats were placed in stainless steel cage units for increasing periods of time (2, 4, 6 and 8 hours) for four consecutive days during the week prior to the study start, and were returned to polycarbonate cages between training periods. Following acclimatization, the rats were placed in the cage units for the duration of the inhalation study except when the cages were changed (weekly), or when rats were weighed (weekly) and monitored for estrous cycle alterations via vaginal lavage and cytology assessment. Rats were provided food and water *ad libitum* throughout the experiment, and were kept on a 12 hour light/dark cycle.

Nose-Only Exposure System (Blood Gas Assessment)

A total of 40 CD® IGS rats (20 male and 20 female), 105-114 days-old, were randomly divided into four groups of 5 males and 5 females. The rats were acclimatized to the nose-only tubes prior to exposure through a process known as restraint training, which involved placing the rats in polycarbonate nose-only tubes for increasing periods of time (9, 30, 60, 90 minutes) for four consecutive days during the week prior to exposures. After each day of restraint training, the rats were returned to their polycarbonate cages, provided food and water *ad libitum*, and kept on a 12 hour light/dark cycle.

Chemicals

Rats were exposed to clean air or mixed atmospheres of CO, CO_2 and O_2. Clean air for the control and exposure system was from an air circulating system using a turbine blower (The Spencer Turbine Co., Windsor, CT) with a room air intake to replace used air through a high-efficiency particulate air filter (HEPA). The mixed atmospheres were generated by adding CO, CO_2, and nitrogen to ambient air from the air circulating system as it entered a chamber. CO_2 was supplied from a dewar of liquefied CO_2 (180 L dewars or 414 pounds liquid per cylinder, Airgas, Lansing, MI). Cylinders of CO_2 (99.99%, 800 cubic feet, 100 pounds CO_2 per cylinder, Weiler Welding Company, Dayton, OH) were used as a backup supply of CO_2 in the event a dewar ran out of liquid CO_2. CO was also supplied from a compressed gas cylinder (99.999%, 4.25 cubic meters, Weiler Welding Company, Dayton, OH). O_2 concentrations were reduced to test conditions by dilution with the appropriate amounts of nitrogen (N_2) provided from a nitrogen generator (Parker Balston Model DB-5, Summit Industries, Inc., Dayton, OH). The nitrogen generator produced 95 to 99% N_2 from in-house compressed air filtered for water and oils.

Inhalation Exposure Chambers

The rats were exposed in one cubic meter whole body exposure chambers (1 m^3, H1000, Lab Products, Seaford, DE) constructed of stainless steel and glass. Separate chambers were used for each of the three test atmospheres, plus three control chambers, for a total of six chambers. Stainless steel cages (R-16, R-24 or R-32, Lab Products, Seaford, DE) were used to contain the rats during inhalation exposures and served as domiciliary housing during the periods of non-exposure. At the beginning of the exposures when the rats were smaller, all rats were housed in R-32 cages. During the breeding period, females were paired with males in R-16 cages and transferred to R-24 cages during gestation and birthing. Stainless steel litter pans were placed under each stainless steel cage to collect the urine and feces. Litter pans were changed daily and cages were changed weekly for the duration of the inhalation exposures.

Nose-Only Exposure System (Blood Gas Assessment)

The test atmospheres were generated in the H1000 whole body exposure chambers described above. The four chambers were used to mix gases in the same concentrations as each of the test atmospheres used during the 90-day exposure period. Rats were exposed using a 52-port Cannon nose-only exposure system (Lab Products, Maywood, NJ). A single NOES was used for all exposures, as a gas mixture was transferred from each H1000 chamber to the NOES at separate times.

Inhalation Exposure Chamber Operation

The inhalation exposure chambers were operated as a push-pull system. Air was pushed into the inlet of the chambers from an air circulating system using a turbine blower (The Spencer Turbine Co., Windsor, CT) with a room air intake to replace used air through a high-efficiency particulate air (HEPA) filter. Air was pulled from the exhaust outlet of the chambers through a manifold connected to an exhaust fan on the roof of the facility.

The target inlet air flow rate in the mixed atmosphere chambers was set to 200 to 250 L/min, providing approximately 12 to 15 air changes per hour. Inlet air flows were a sum total of the clean air, CO flow, CO_2 flow and N_2 flow. Inlet air flows were controlled by a manually operated gate valve. Inlet air flows were monitored by mass flow monitor (Model HFM-200 LFE, Teledyne-Hastings Instruments, Pittsburgh, PA) connected to a laminar flow element (Model HFM-200 LFE, Teledyne-Hastings Instruments, Pittsburgh, PA). Each of the mass flow monitors was connected to a four-channel power supply (Model THPS-400-115, Teledyne-Hastings Instruments, Pittsburgh, PA).

The inlet air flow for the control chamber was set to a target flow rate of approximately 408 L/min (approximately 24.5 air changes per hour) to dilute the CO_2 concentrations produced by

the collective exhaled breath from the animal load. The higher flow rate to the control chamber resulted in somewhat lower humidity conditions for rats in the control group in comparison to the rats from the dose groups, but this difference was not expected to affect study results.

The chamber exhaust flow for the mixed atmosphere exposure chambers was adjusted with a manually operated gate valve to maintain a slight negative pressure relative to the room during the exposure to prevent the test atmosphere from entering the laboratory area in the event of leaks. The control chamber's exhaust flow was adjusted to maintain a slight positive pressure relative to the room to minimize the possibility of a contaminant entering the chambers.

The static pressure of each inhalation chamber was determined using both a magnehelic gauge (Model 2304, Dwyer Instrument Co., Michigan City, IN) with a visual display and an electronic sensor (Model ZPS-05-SR09-EZ-ST-D, Building Automation Products, Inc., Gays Mills, WI).

Nose-Only Exposure System (Blood Gas Assessment)

Each H1000 inhalation exposure chamber was operated as described above, with the exhaust flow manually adjusted with a gate valve to maintain positive pressure relative to the laboratory during the exposure period to push the test atmosphere from H1000 into the inlet of the NOES. The exposure atmosphere flowed from each pressurized H1000 (+1.0 inch H_2O) at a total flow rate of approximately 26 L/min through the inner plenum and out through the delivery nozzles into the breathing zone of each animal at approximately 0.5 L/min per open port. The nose-only exposure system was fitted with a differential pressure gauge to monitor static pressure at an open port. The outer plenum of the nose only exposure system carried the animal's exhaled breath and excess test atmosphere to an exhaust set at a flow rate of approximately 26.0 L/min. The NOES operated as a push-pull system where the air supply was positive and the exhaust flow was negative. The exhaust was set at the target flow rate and the supply was adjusted to

maintain a static pressure (Magnehelic® Gage, Dwyer Instrument Co., Michigan City, IN) in the range of -0.05 to -0.10 inches of H_2O for the exposures. Nose-only tubes with a urine and feces cup (CH Technologies, Westwood, NJ) were used for animal containment during exposures. Medium or large tubes (CHT 249 or 250) were used for the smaller females and extra-large tubes (CHT 2500) were used for the males.

Temperature and Humidity

Temperature and relative humidity were measured by a temperature and relative humidity probe (Model HF532WB6XD1XX, Model HC2-S, Rotronics Instruments, Inc., Hauppauge, NY) located inside of each exposure chamber. The target temperature inside each chamber was maintained between 17.5 to 26°C and the target relative humidity was maintained between 30 and 70%.

Atmosphere Generation

All test chemical gases for the mixed atmospheres were metered by mass flow controllers (Model HFC-202, Teledyne-Hastings Instruments, Pittsburgh, PA) at flow rates appropriate to maintain target concentrations of mixed atmospheres for each of the target doses. Each of the mass flow controllers were connected to a four-channel power supply (Model THPS-400-115, Teledyne-Hastings Instruments, Pittsburgh PA) and manually adjusted to the appropriate flow rate for the target concentrations. Figure 1 shows a diagrammatic representation of the whole body exposure system.

Nose-Only Exposure System (Blood Gas Assessment)

The mixed atmospheres used in the NOES were generated inside of the whole body (H1000) exposure chambers, as per the methods described above. A small amount of carbon dioxide was added to the test atmosphere for the control group to simulate the minimal concentrations

generated by the exhaled breath of chambered rats during the 90-day exposure. Figure 2 shows a diagrammatic representation of the nose-only exposure system.

Test Atmosphere Monitoring

The mixed gas test atmosphere of each of inhalation chamber was monitored continuously with a multiple gas analyzer (Model VA-3113, Horiba Instruments, Inc. Moon Township, PA). Each instrument contained a magnetopneumatic (MP) sensor for O_2 measurements and two non-dispersive infrared analyzers (NDIR) for CO and CO_2 measurements. The oxygen sensor for the high-dose chamber was damaged by moisture on exposure day 45 and was provisionally replaced with a microfuel cell oxygen analyzer (Model CO6689-B1, Teledyne Instruments, City of Industry, CA) until another multiple gas analyzer was installed for the high-dose chamber on study day 57. The sensor failure resulted in 12 days of suspect oxygen data for the high-dose chamber. A sample line dryer (Model No. MD-110-125-4 388 1010, PermaPure, Toms River, NJ) was added to each sample line where it exited the inhalation chamber to prevent moisture from damaging other sensors. Each instrument was calibrated using a N_2 dilution manifold and varying amounts of calibration gases (Airgas, Dayton OH): 500 ppm CO in N_2, for the CO NDIR, 5% CO_2 in N_2 for the CO_2 NDIR, and room air (20.9% O_2) for the O_2 MP and microfuel cell. Instruments were zeroed using N_2.

Nose-Only Exposure System (Blood Gas Assessment)

The mixed gas atmosphere used in the NOES for each group was monitored continuously using two of the multiple gas analyzers described above. One analyzer monitored the atmosphere in the H1000 exposure chamber. The second analyzer was used to monitor the gases introduced into the nose-only exposure system to validate the true exposure. Both of the instruments were calibrated daily for expected concentration ranges.

Automated Alarm System

The monitoring sensors for the key environmental parameters of temperature, relative humidity, airflow, CO concentration, CO_2 concentration and O_2 concentration within the inhalation chambers were electronically connected to an alarm system (Model FGD-2000, Sensaphone, Aston, PA) that automatically contacted assigned study personnel if any parameters fell outside of acceptable ranges. This system also recorded data every 30 minutes to serve as back-up data to the primary data recording system.

Exposure Data Collection

Data from the monitors and flow sensors were collected via automation by a computer using data acquisition software (LabView Software v.10.0, National Instruments, Austin, TX). Data were collected every 10 seconds for temperature, humidity, supply air flow, CO concentration, CO_2 concentration, O_2 concentration, CO flow rate, CO_2 flow rate, N_2 flow rate, and static pressure for each mixed atmosphere group. The 24-hour data report for each dose group was collected from approximately 0900 until 0900 the following day. Periods when the chambers were opened for animal husbandry and animal procedures or power failures due to significant weather were included in daily averages to reflect the actual average exposure concentrations experienced by the rats. However, data were eliminated from the daily average for equipment malfunctions (e.g., excess humidity in sample line or oxygen sensor failure). At 0900 of each day, the average, standard deviation, minimum values, maximum value and the total number of data values were calculated. The collective daily averages were used to calculate the average of daily averages, standard deviation of daily averages, minimum daily average, maximum daily average and number of daily averages.

Data collection for the three control chambers was handled differently, since these chambers had a common sample line to a single monitor. Therefore, the mean concentrations for CO,

CO_2 and O_2 represent the average of daily averages for the three control chambers collectively. Data for temperature, humidity, supply airflow, and static pressure for each of the three control chambers were collected every 10 seconds, with the individual mean values averaged and the standard deviation calculated (n=3) for this report.

Nose-Only Exposure System (Blood Gas Assessment)

H1000 data were collected as described above. Additional data were collected every 10 seconds for the NOES concentrations of CO, CO_2 and O_2. The exhaust flow rate and NOES static pressure were recorded manually in the data notebook approximately every 30 minutes during the exposure. At the close of each of the four exposure days (one day per test atmosphere), the average, standard deviation, minimum value, maximum value and the total number of data values were calculated for each environmental exposure parameter.

Study Day

A study day was defined as a 24-hour period generally from approximately 0900 until 0900 the following day. The study days were numbered consecutively from 1 to 109 corresponding to the first day when the control group was loaded into the control chamber until the last day when the high exposure group was removed from the high-dose chamber. Exposures were interrupted each day for approximately 15-60 minutes to replenish the animal feed, inspect or change equipment, observe rats for health and clinical signs, measure animal weights, change out waste collection trays, and take biological samples. The weight of each rat was measured daily to the nearest gram using an electronic scale (Sartorius Model 1507, DWS, Inc., Elk Grove, IL) up until the mating period began (exposure day 70). Biological samples included the taking of blood via tail vein sticks and vaginal fluids via lavage methods. The parameters measured for the parental generation (P1), first generation offspring (F1) and second generation offspring (F2) during the exposure and post-exposure periods are listed in Table 1.

Nose-Only Exposure System (Blood Gas Assessment)

A study day for the blood gas assessment was defined as the approximate 90 to 105 minute exposure period for all rats. The rats were placed on the NOES at 10 or 15 minute intervals, staggering males and females. The exact time between the placement and removal of a rat on the NOES was determined by the time it took to draw blood from the tail vein. Following 90 minutes of exposure to one of the mixed gas test atmospheres via the NOES, 0.5 mL of blood was collected from each rat via a tail stick with a 22 gauge, heparinized needle into a 2.0 mL Eppendorf tube containing 0.125 mL heparin. The blood was analyzed with a Gem Premier 4000 analyzer (Instrumentation Laboratory, Bedford, MA) to measure COHb, pCO_2, pO_2, and total CO_2. Samples were run in duplicate and analyzed within 5 minutes of being collected. Once the blood was collected, the exposures were discontinued and the rats were euthanized.

Serum Hormone Levels

Blood samples from P1 females for hormone and vitamin D analysis were obtained via tail vein stick on day 69 of exposure, approximately 24 hours prior to being paired for mating. Blood samples from males were obtained via cardiac puncture during necropsy, immediately following 90 days of exposure. Serum was separated by centrifugation at 4000 rpm for 10 minutes. Serum samples were frozen, and stored at -20^{o}C until the hormone analysis was performed with standard ELISA for Follicle Stimulating Hormone and Luteinizing Hormone (Shibayagi Co. Ltd, Ishihara, Japan), and for vitamin D (Cusabio Biotech Co., Ltd, Newark, DE), or with a Multi Spot Assay System (Meso Scale Discovery, Gaithersburg, MD) for DHEA, estradiol, progesterone, and testosterone. Analyses were completed using the manufacturer's instructions, as written.

Estrous Cycle Monitoring

P1 Generation

Estrous cycle phases were categorized for randomly selected female rats (eight per exposure group) by the employment of vaginal lavage methods previously published (Marcondes et al., 2002). Dose group comparisons to controls were based on the proportion of days that rats were observed in each of the estrous cycle phases during the 11-day estrus observation period. The metestrus and diestrus phases were combined into a single category, since these phases are very difficult to differentiate. The evaluation period began following a full estrous cycle under exposure conditions. If the categorization was ambiguous (e.g., designated as positive for both proestrus and estrus phases), then each phase category was scored as an observation of 0.5 rat-days. If an insufficient number of cells were recovered to categorize an estrous cycle phase, then the data were excluded; as a result, the number of rat-days sometimes varied between groups. Proportional differences between the dose groups and the controls were evaluated for statistical significance ($\alpha = 0.05$) for each of the estrous cycle phases.

F1 Generation

The estrous cycles of 32 F1 female rats (eight per exposure group) chosen for mating were determined by the same procedures as described above for P1 generation females, with the exception that the F1 rats were monitored for 14 consecutive days prior to mating.

Mating and Monitoring of Pregnancy and Offspring

P1 Generation

Following 70 consecutive days of exposure, the rats from each exposure group were randomly sorted into mating pairs and placed into common exposure cages for mating. The waste trays of the exposure cages were checked daily by study personnel for evidence of mating (copulatory plugs). The date on which the copulatory plug was discovered was designated Gestation Day 0

(GD 0). When a copulatory plug was discovered, the mating pair was separated, and placed into separate exposure cages. If no evidence of mating was found for the mated pair by day 7, the mating was recorded as a “failure”, and the male was removed to a separate cage to continue exposure for 90 days. P1 females for which no copulatory plug was identified were weighed every three days for two weeks and monitored for any significant weight changes to determine instances of unrecognized pregnancy. The ultimate test of successful mating was the birth of a litter. Females failing to reach parturition after 105 days of exposure were designated non-breeders, removed from the exposure chamber, and necropsied, with their tissues prepared for histopathological examination.

To minimize handling of pregnant dams, weight measurements were not collected from GD 14 to parturition. Pregnant dams were removed from the exposure chambers on GD 19 and then transferred to single poly-carbonate cages with wood chip bedding located in the vivarium, where they were monitored twice daily for evidence of birth. Post-natal day “zero” (PND 0) was designated as the date on which the first pup of a delivery was discovered. Litter size (number of living and stillborn pups), sex distribution, and litter pup weight for males and females, were recorded no later than PND 1. The general physical condition of the litter (dam and pups) and number of gross malformations per litter were assessed twice daily from PND 1-4 and daily thereafter. No attempt was made to augment or supplement maternal care at any time during the study. All deceased pups found in cages were examined for gross defects and necropsied in an attempt to determine the cause of death. On PND 4, litters were standardized to eight pups by random selection, but in as close to an equal male and female ratio as possible.

Randomly selected P1 animals underwent neurobehavioral assessments after mating was completed (32 males), or after the weaning of their F1 pups (32 females).

F1 Generation

Upon maturity (>70 days) 128 F1 rats were randomly sorted into mating pairs within the same exposure group as their parents. Special care was taken to ensure that siblings were not paired for mating. The rats were placed in wire-bottom, polycarbonate mating cages. The mating cages were checked daily by study personnel for evidence of mating (copulatory plugs). The date on which a copulatory plug was discovered was designated GD 0. When a copulatory plug was discovered, or at the end of the 14-day mating period, the pair was separated, with each male and female placed into separate polycarbonate cages with appropriate bedding. Females that did not produce evidence of mating were weighed every third day for two weeks to monitor for significant weight changes consistent with pregnancy. The ultimate test of successful mating was the birth of a litter. To minimize handling of pregnant dams, weights were not collected after GD 14. Dams failing to reach parturition were designated as non-breeders and necropsied, with tissues prepared for histopathological examination.

Beginning on GD 19 dams were monitored twice daily by study personnel for evidence of birth. PND 0 was designated as the date on which the first pup of a delivery was discovered. Upon complete delivery, litter size (number of pups, living and stillborn), sex distribution, and litter pup weight for male and female groups, were recorded no later than PND 1. The general physical condition of the litter (dam and pups) and number of malformations per litter (pups only) were also assessed twice daily by study personnel from PND 1-4, and at least daily thereafter. No attempts were made to augment or supplement maternal care at any time during the study. All deceased pups were examined for gross defects, and necropsied in an attempt to determine the cause of death. On PND 4, F2 litters were standardized to eight pups by random selection, but in as close to an equal male and female ratio as possible.

Randomly selected F1 animals underwent neurobehavioral assessments after mating was completed (32 males), or after the weaning of their F2 pups (32 females).

F1 and F2 Rats

Eight litters (four male pups and four female pups) from each mating group underwent neuro-behavioral assessments from PND 3 to PND 8. Dams were allowed to nurse and care for their selected F1 and F2 litters through PND 20. F1 and F2 offspring were weighed and weaned on PND 21, after which they were either necropsied, or randomly sorted by sex into small groups of (2-4) animals from the same litter and housed until required for breeding, adult neurobehavioral assessments, and adult necropsy. F1 and F2 offspring chosen for maturation remained group housed to PND 40, at which point they were moved to single housing until reaching sexual maturity (>PND 51). Necropsies were performed on F1 and F2 adults following completion of adult neurobehavioral assessments, with all tissues prepared for histopathological examination.

Necropsy

On the day of the necropsy, male and female animals were anesthetized by CO_2 overdose until unresponsive, after which blood was sampled via cardiac puncture. After blood collection, the rats were decapitated and all target organs were harvested for analysis. Blood/serum was collected and processed for clinical chemistry and hematology analyses following standard laboratory procedures. Target tissues were harvested using standard necropsy methods. Blood samples were frozen at - 20^{o}C until processed for analysis. Tissue samples were fixed in formalin or Bouin's solution depending on tissue type.

Hematology

Complete blood count (CBC) analysis was performed on 40 µL samples of whole blood taken from each animal using a blood analyzer (HemaVet® HV950, Drew Scientific, Inc., Waterbury,

CT). Parameters measured were: number per µL of white blood cells (WBC), red blood cells (RBC), lymphocytes (LY), monocytes (MO), neutrophils (NE), eosinophils (EO), basophils (BA), % LY, % MO, % NE, % EO, % BA, and platelets (PLT); % hematocrit (HCT); g/dL of hemoglobin (HB) and mean corpuscular hemoglobin concentration (MCHC); mean corpuscle volume (MCV); mean corpuscular hemoglobin (MCH); and, red blood cell distribution width (RDW).

Serum Chemistry

Serum chemistries were measured using a chemistry analyzer (VetTest® 8008, IDEXX Labs, Inc., Westbrooke, ME) and electrolyte analyzer (VetLyte®, IDEXX Labs, Inc., Westbrooke, ME). A 100 µL sample of serum from each animal was analyzed for total protein (TP), albumin (ALB), alkaline phosphatase (ALKP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), cholesterol (CHOL), creatinine kinase (CK), creatinine (CREA), globulin (GLOB), glucose (GLU), total bilirubin (TBIL), triglycerides (TRIG), and major electrolyte concentrations (Na^+; K^+; Cl^-).

Tissue Histopathology

Select tissues and organs were fixed in formalin, properly sealed and packaged, and express shipped to an external histopathology laboratory (Seventh Wave Histology Laboratory, 743 Spirit 40 Park Drive, Chesterfield, MO) contracted to conduct the histopathological analysis. The following tissues were prepared/submitted for evaluation: adrenal glands, brain (basal ganglia, hippocampus and hypothalamus), heart, kidneys, liver, mammary glands (females only), pancreas, pituitary gland, spleen, male reproductive organs (testes and epididymides) and female reproductive organs (ovaries, uterine horns and uterus). The external histopathology laboratory trimmed the tissues, embedded, cut, mounted and stained the tissues for microscopic examination by a pathologist. The remaining permanent, formalin fixed, paraffin embedded, 5 micron, hematoxylin and eosin stained tissue sections were archived at NAMRU-Dayton.

The pathologist at the contract histopathology laboratory evaluated six individual consignments of rat tissues: (1) directly exposed P1 adults; (2) F1 juveniles; (3) F1 adults that were not selected for breeding; (4) F1 adults that were randomly selected for breeding; (5) F2 juveniles; and, (6) F2 adults. The tissues from eight female rats and eight male rats, which were randomly selected to represent each dose and control group from each consignment were evaluated histopathologically. The tissues from mated rats that could not produce offspring were also evaluated histopathologically to determine if the dysfunction resulted from a dose-related effect. In addition, formalin-fixed testes from 16 P1 males (eight controls and eight rats from the high-dose group) were examined to evaluate staging in spermatogenesis. The testes were infiltrated and embedded in glycol-methacrylate, sectioned, stained with PAS-hematoxylin, and examined microscopically. Macroscopic counting of uterine implantation sites was also performed for 16 P1 dams (eight controls and eight dams from the high-dose group). The formalin-fixed uteruses were grossly examined with a bright light under magnification to detect the implantation sites. The total number of implantation sites in the two uterine horns was recorded for each dam.

The gross pathology at necropsy, and during the course of the in-life portion of the study was performed by LTC Deidre Stoffregen (VC, USA, DACVP) from NAMRU-Dayton, WPAFB, OH.

Neurobehavioral Assessment

Following 90 days of whole body submarine atmosphere exposures and “in-chamber” mating, neurobehavioral assessments were conducted on exposed (P1 generation) males and females and two generations of their offspring (F1 and F2). Testing procedures were similar to previous developmental studies performed in this laboratory (McInturf et al., 2008; Arfsten et al., 2009). A detailed schedule of neurobehavioral testing is contained in Table 2.

On PND 2-3, 32 dams from each of the exposed (P1) and first generation (F1) groups were tested for maternal retrieval latency (Hahn and Lavooy, 2005). On PND 4, litters were culled to eight pups (four males and four females) and inspected for physical birth defects; non-selected pups were euthanized via CO_2 overdose. The random selection of pups attempted to maintain an equal sex litter ratio, when possible. After the first cull, additional developmental testing was conducted, examining righting reflexes (Pellis et al., 1991) on PND 4-5 and separation distress, as measured by ultrasonic vocalization (Bekkedal et al., 1999; Hahn and Lavooy, 2005) on PND 7-8. Following weaning on PND 21, litters were culled to a single male and female from each parental exposure group, which were retained for further testing after sexual maturity.

Adult neurobehavioral testing for all generations of animals included motor activity assessments and water maze navigation using a modified Morris water maze methodology (Morris, 1984; Buccafusco, 2001). Testing of exposed P1 males was performed within 21 days post-exposure. Testing of exposed P1 females was performed within 21 days post weaning. Testing of adult F1 and F2 offspring was performed over a 1 month period between PND 48-67 for rats not selected for breeding and between PND 68-84 for rats randomly selected for breeding.

Assessments in Female Parents (P1 & F1 Females)

Maternal Retrieval

Instinctual maternal response was evaluated using the test of maternal retrieval on PND 2-3. The dam was momentarily transferred to an empty cage while three pups were taken from the nest and moved to the opposite end of the cage. The dam was immediately placed back into the cage. The time period (latency) for the dam to retrieve all three pups and return them to the nest was recorded in seconds using a standard stopwatch. If the dam had multiple nests, a single nest was created. Dams meeting this criterion, and with failure to retrieve, were retested with the nest moved at the opposite end of the home cage. Tests “timed-out” after 5 minutes.

Assessments in F1 & F2 Pups

Righting Reflex

Development of early motor coordination was assessed in the pups with the test of righting reflex on PND 4 or PND 5. Individual pups were placed in a supine position on a Plexiglas platform. The pups were gently held down by positioning an index finger along the abdomen. The finger was removed and the latency for the pup to roll over and obtain the prone posture with all four paws on the platform was timed. The procedure was immediately repeated two more times, for a total of three consecutive tests, and the scores were averaged for statistical analyses. If a pup failed to right within 60 seconds, it was classified as "timed-out".

Separation Distress

Emotionality was measured in the pups by recording the ultrasonic distress vocalizations (USV) emitted upon separation from the dam and littermates on PND 7 or PND 8. Tested dams and litters were taken to a room separate from the home room where individual pups were placed into an enclosed sound attenuating cubicle which contained a steel rod floor chamber with an ultrasonic vocalization detector (ANL-9371, Med Associates, Inc., St. Albans, Vermont). The chamber testing was conducted in the dark at 21°C. Each pup was individually placed into the chamber and allowed to move about freely. The detector was started once the door was shut.

The number of distress vocalizations occurring at 40 kHz range, a frequency unique to pup distress calls (Blumberg and Alberts, 1991) was recorded for 60 seconds. Recordings within the 39-41 KHz range occurred over two bands. Band 1 had a threshold setting of 30dB and band 2 had a threshold setting of 40 dB. These band width settings were low enough to detect vocalizations and high enough to prevent background noise detection. Since the average vocalization detection levels were closer to the 30dB threshold band, the 30dB band was used for vocalization analyses. The chamber was sanitized between tests to remove olfactory cues.

Assessments in Male and Female Parents and Adult F1 & F2 Offspring

Motor Activity (MA)

Gross locomotor movements and exploratory behavior were evaluated in the parents and selected adult offspring using a photobeam activity system (PAS) and associated software (San Diego Instruments, San Diego, CA). Rats were individually placed in clear plastic open field boxes (16" W x 16" D x 15" H) with horizontal and vertical photobeam frames that automatically recorded beam breaks using the PAS software. The activity chambers had photocells aligned 2.54 cm (1 inch) apart to differentiate between horizontal movement, vertical rears, and fine (stereotypic) movements. The apparatuses were located in a room with white noise generated at 68dB to mask ambient room levels of ~65dB; also, the light source was adjusted to a low illuminating setting of 30 lux. To begin the test, animals were placed in the center of the open field and left uninterrupted for the duration of the 30-minute test session. Between each test and each animal, urine and feces were removed and the open fields were washed with a solution of 10% ethanol to remove any olfactory cues that may have been left behind. The measures recorded include distance traveled (cm), active time/resting time (sec), average speed (cm/sec), number of fine beam breaks (stereotypical), number of rears, and percentage of time in center vs. perimeter.

Water Maze Navigation

Water maze navigation was used to evaluate motor coordination, spatial learning and memory (Morris, 1984; Voorhees and Williams, 2006) in parents and a portion of their adult offspring. The maze construct used was a dark blue plastic tank with a 183 cm diameter and 30 cm height (San Diego Instruments, San Diego, CA). The tank was filled to 20 cm (8 inches) from the top with water that was maintained at 22°C to 25°C. A 10 cm square clear escape platform large enough for the animal to stand on was attached to the floor of the tank, but submerged 1" below

the surface of the water. Shiny, patterned visual cues of different shapes (i.e. triangle, square, circle, etc.) were mounted on boards outside of the tank and designated for each quadrant.

During training, the animal was placed in the water facing the wall at one of three locations distal from the escape platform. Throughout the training, animals were pseudo-randomly placed into the different quadrants so that all quadrants equally served as start zones and no obvious pattern could be learned. The animal was allowed to swim until reaching the escape platform or until the 90 second time-out. The animal was removed from the tank, dried with a towel, and given a 15 second rest period before the next trial. Animals were trained three trials per day for five days until they could consistently swim to the platform in less than 20 seconds. On the sixth day, the platform was removed from the tank and a single 90-second probe trial test was administered for each animal. For the probe trial, the animal was placed in the most distal quadrant in relation to the previous platform location. The total distance (cm) the animal swam and the latency (sec) to find the platform were electronically recorded for 90 seconds using a SMART tracking system and the water maze software (San Diego Instruments, San Diego, CA). On the probe trial day, the percentage of time spent in the previous platform quadrant and the number of crossings over the previous platform location were also recorded.

Results

Environmental Parameters

The whole body inhalation exposure system and the nose-only exposure system performed as designed and demonstrated the laboratory's capability to control test conditions within the parameters specified by the study protocol. Data of the environmental parameters inside of the H1000 inhalation chambers during the 90-day exposure period are provided in Table 3. Data of the environmental parameters inside of the H1000 inhalation chambers while mixing the test atmospheres used to perform the 90-minute blood gas exposures are provided in Table 4. Data

of the environmental parameters in the gas mixtures transferred to the NOES during 90-minute exposures to conduct blood gas analysis are provided in Table 5. Data for the test chemical flow rates used in the 90-day exposures are provided in Table 6. Data for the test chemical flow rates used in the 90-minute exposures for the blood gas measurements are provided in Table 7.

Deaths

P1 Generation

No rats died during the 90-day exposure; however, one female from the mid-dose group was removed from the study due to a mandibular occlusion. The rat was unable to eat and was euthanized according to protocol. Since no other rats experienced this issue, it was attributed to a congenital defect or incisor overgrowth, and not a result of the exposure.

One dam from the mid-dose group was found dead in her cage on PND 4. Aside from a smaller than average litter size (six pups), nothing was remarkable about the dam, and all six pups were alive when the dam was found. The necropsy showed no internal defects, and death was attributed to overt stress from parturition.

F1 Generation

Two males from different mid-dose group litters were found dead in their cages on PND 42 and PND 71, respectively. Necropsy revealed no apparent causes of death. The litters from which these rats originated were unremarkable and all littermates survived until euthanized.

One dam from the low-dose group as found dead in her cage 12-18 hours after parturition of 19 pups. All pups were alive at the time of discovery and were observed to be in good condition. Necropsy revealed no internal damage, and the death was attributed to stress of parturition. No other female rats experienced this issue.

Blood Gases

After 90 minutes of inhalation exposure via the NOES, mean carboxyhemoglobin (COHb) levels in the rats from the control, low-, mid- and high-dose groups were 1.39 ± 1.43 %, 1.57 ± 0.47 %, 1.97 ± 0.26 % and 11.36 ± 1.40 %, respectively. These levels are consistent with the published experimental values and with physiologically based pharmacokinetic models for the four carbon monoxide concentrations tested (Andersen et al., 1991; Benignus and Annau, 1994). These data are indicators of saturated blood gas levels of carboxyhemoglobin expected for each of the dose groups evaluated during the 90-day study. Additional blood gas parameters (pCO_2, pO_2, and total CO_2) varied greatly with no dose-related pattern and are not included in this report.

Body Weights/Body Weight Gains

Statistical differences in animal weight gains for exposed animals were assessed using Kruskal-Wallis analysis of variation (ANOVA) with Conover-Inman corrections for pair-wise comparisons. The critical values that are reported are the degrees of freedom, error degrees of freedom, the *H* statistic, and the associated p-value. Pair-wise p-values are also reported. Only significant values are presented in complete format. The U.S. EPA Benchmark Dose Technical Guidance (EPA/630/5-00/001, 2000) was also used to determine the biological significance of average weight changes between dose groups and controls, with a weight change difference between groups at ≥ 10% considered to be significant.

The average weight change for exposed animals and controls were calculated for each group from the differences between each group's average weight at 10 weeks and 18 weeks of age. Weight change data are provided in Tables 8 and 9. For female rats, the average weight gain over this 56-58 day observation period for each of the three dose groups was not significantly different from controls. However, the average weight gain in exposed male rats from the mid- and high-dose groups were significantly lower compared to controls ([H (3, 32.46) = 0.001, KW],

C < M, p = 0.003, C < H, p = 0.001, Table 9), which appears to be a dose-related effect. Data collected in Phase 2 showed similarly reduced weight gain in the males from the mid- and high-dose groups after 28 days of exposure (NAMRU-D-12-03). The biological significance of this finding is marginal based on the U.S. EPA criteria, with the largest weight change difference measuring 9.7% between high-dose group males and controls.

Serum Hormones

A screen of serum hormone and vitamin D levels was conducted to identify possible endocrine disruptions from exposure that could have an impact upon reproduction. Statistical differences between average hormone levels in exposed animals from the dose groups and controls were assessed using the student t-test. The critical values reported for the t-tests are the degrees of freedom, the t-statistic, and the associated p-value.

The only statistically significant difference identified was the higher luteinizing hormone (LH) levels found in exposed females from the high-dose group, prior to mating, in comparison to controls ($t(10) = 3.27$, $p = 0.008$, Table 10). However, this difference is not considered to be dose-related, as these hormone levels were within the normal range and most likely reflect the random distribution of ovulating females within small sized populations. All other hormone concentrations tested, as well as vitamin D levels, were within normal ranges and consistent between the high-dose group and controls.

Estrous Cycles

The difference between the proportions of time spent in each estrous cycle phase for each dose group in comparison to controls were compared using the Pearson Chi-square test (X^2, $\alpha=0.05$).

There were no indications that exposures to the three test gas mixtures had any effect upon the estrous cycle, since female animals from the dose groups were observed to have estrous cycle phase proportions that are not statistically different from the control animals (Table 11).

Mating and Monitoring of Pregnancy and Offspring

The proportional differences between dose groups in comparison to controls for dams exhibiting evidence of pregnancy (mating index) and for pregnant dams producing live offspring (gestation index) were determined using the Pearson Chi-square test (X^2, $\alpha = 0.05$). If a difference among the groups was identified, further comparisons between groups were conducted using Fisher's exact test. Other endpoints in Tables 12 and 13 were evaluated using one-way analysis of variance (ANOVA). If the data failed the Shapiro-Wilk normality test, a Kruskal-Wallis one-way ANOVA on ranks was completed. The dose groups with significant effects were identified using Dunn's (post hoc) method for multiple comparisons. The critical values reported for the ANOVAs are the main effect degrees of freedom, error degrees of freedom, F-ratio, and the associated p-value. For X^2 tests, the critical values reported are degrees of freedom, sample size and the X^2 statistic. Pair-wise p-values are also reported, as well as p-values > 0.05 for certain critical study measures; however, only statistically significant values are presented in complete format.

P1 Generation

Gestation (Table 12) and parturition (Table 13) data indicate that exposure to the test submarine atmospheres was not a significant factor affecting any of the measures of reproductive success, including: mating success (indicated by the presence of a copulatory plug or a delivery of pups); the proportion of pregnant dams producing living offspring (gestation index); the fraction of pups born alive (live-born index), the length of gestation; litter size; and, the sex ratio (male fraction of

live pups). Also, a numerical comparison between uterine implantation sites and litter size for a random sample of exposed dams showed no statistically significant resorption rates (Table 14).

F1 Generation

Developmental data for first generation offspring, who were exposed *in utero* to GD 19, indicate no statistically significant differences based on parental exposures. Data included: viability at PND 4 (viability index) and PND 21 (lactation index); the average day to open eyes and ears; and, the average pup weights at PND 0 and PND 21 (Table 13).

Mating success for the first generation offspring also remained unaffected by parental exposure. However, the proportion of pregnant dams producing live born offspring (gestation index) varied among dose groups ([X^2= 11.346, 3], p = 0.010, Table 12) and was increased in the mid-dose ([Fisher's exact test, p = 0.024) and high-dose (Fisher's exact test, p = 0.024) groups compared to controls. Other significant differences among groups were found for litter size (Kruskal-Wallis one-way ANOVA on ranks, H = 8.396, 3, p = 0.039, Table 12) and the number of live pups per litter (Kruskal-Wallis one-way ANOVA on ranks, H = 8.978, 3, p = 0.030, Table 12); however, no significant differences between groups could be identified in pair-wise tests (Dunn's Method) for these two factors. Other reproductive endpoints that were unaffected by exposure included: gestation length; the stillbirth index; the proportion of live born offspring to the number of uterine implantation sites; and, the sex ratio.

F2 Generation

The only developmental data for unexposed F2 offspring that indicated a statistically significant difference between groups was the average time to open eyes and ears (Kruskal-Wallis one-way ANOVA on ranks, H = 12.318, 3, p = 0.006, Table 13); however, pair-wise tests (Dunn's Method) indicated that the main differences were between the low-dose and high-dose groups

($Q = 2.837$, $p < 0.05$), with no significant differences identified between the dose groups in comparison to controls. Other developmental indicators were unaffected by parental group, including: viability at PND 4 (viability index) and PND 21 (lactation index); and, average pup weights at PND 0 and PND 21.

Tissue Weights

Organ weight differences were determined by an analysis of covariance (ANCOVA). Effects examined were the dose group and the status of paternal exposure (exposed or unexposed). For all tests performed, weight was considered the covariate. Data were checked with Levene's test for equality of variances. Homogeneity of slopes (HOS) was determined by looking for non-statistically significant interaction terms. If the slopes were not significantly different from each other for both factors, then the ANCOVA was run. If the slopes were significantly different, then the McSweeney-Porter method was used to convert the response and covariate into ranks before the ANCOVA was run. The critical values that are reported are the main effect degrees of freedom, error degrees of freedom, F-ratio, and the associated p-value.

P1 Generation – Exposed Females (195-214 day old Breeders)

There were no statistically significant differences between the mean weights of organs (brain, heart, kidneys, spleen, liver and ovaries) of tissues taken from female P1 rats at necropsy, in comparison to controls (Table 15). Female rats were euthanized 5-8 weeks after a continuous 90-105 day exposure to allow for weaning and the completion of adult neurobehavioral tests.

P1 Generation – Exposed Males (163-172 day old Breeders)

There were no statistically significant differences observed between the mean organ weights of the brain, heart, spleen, liver and testes tissues taken from exposed P1 male rats at necropsy, in comparison to controls (Tables 16). However, there were significant decreases in the mean

kidney weights of male rats from the dose groups compared to controls (Left Kidney: [F (3, 29) = 3.97, p = 0.017], C > L, p = 0.023, C > H, p = 0.042; Right Kidney: [F (3, 29) = 8.74, p = 0.001], C > L, p = 0.002, C > H, p = 0.001, Table 16). Male rats were euthanized 3-5 weeks following a continuous 90-day exposure to allow for the conducting of adult neurobehavioral tests.

F1 Generation (PND 25-36)

There were no significant differences observed between the mean organ weights (brain, heart, kidneys, spleen, liver, and ovaries or testes) of tissues taken from the F1 juvenile offspring of exposed parents at necropsy, in comparison to controls (Tables 17 and 18). The F1 juvenile female and male rats were exposed *in utero* to GD 19 and were euthanized following weaning and the conducting of early development neurobehavioral tests.

F1 Generation (PND 94-105)

There were no significant differences observed between the mean organ weights (brain, heart, kidneys, spleen, liver, and ovaries or testes) of tissues taken from the F1 adult offspring of exposed parents at necropsy, in comparison to controls (Tables 19 and 20). The F1 adult female and male rats were exposed *in utero* to GD 19 and were euthanized following the attainment of sexual maturity and the completion of adult neurobehavioral tests.

F1 Generation – Randomly Selected Breeders (PND 114-126)

There were no significant differences observed between the mean organ weights (brain, heart, kidneys, spleen, and ovaries or testes) of tissues taken from eight male rats and eight female rats that were randomly selected as breeders from F1 offspring, in comparison to controls (Tables 21 and 22). There were also no significant differences in mean liver weights between the dose groups and controls for the F1 male breeders. However, there were significantly higher liver weights in the female dose groups of F1 breeders compared to controls ([F (3, 27) =

11.72, p = 0.001], C < L, p = 0.001, C < H, p = 0.004, Table 21). F1 breeders were euthanized following the weaning of F2 pups and the completion of adult neurobehavioral tests.

F2 Generation (PND 23-28)

There were no statistically significant differences observed between the mean organ weights of the brain, heart, kidneys, liver, and ovaries or testes taken from the unexposed, juvenile, F2 offspring of parents exposed *in utero* to GD 19 in comparison to controls (Tables 23 and 24). There were several low spleen weights among the dose groups compared to controls for both female and male rats; however, the only statistically significant difference was between the female mid-dose group compared to controls ([F (3, 27) = 4.28, p = 0.014], C > M, p = 0.028, Table 23). The F2 juvenile rats were euthanized following their weaning and completion of early development neurobehavioral tests.

F2 Generation (Females: PND 93-101; Males: PND 86-99)

There were no significant differences observed between the mean organ weights (brain, heart, kidneys, spleen, liver, and ovaries or testes) of tissues taken from the adult, unexposed, F2 offspring of parents exposed *in utero* to GD 19, in comparison to controls (Tables 25 and 26). The F2 adult female and male rats were euthanized following attainment of sexual maturity and the conducting of adult neurobehavioral tests.

Hematology

Differences in hematology values between the dose groups were determined by a one-way ANOVA when data were found to be normal or by a non-parametric one-way Kruskal-Wallis (KW) test when data normality failed the Shapiro-Wilk test ($p < 0.05$). Pair-wise comparisons for the ANOVAs were performed using Tukey–Kramer procedures, or by using Conover-Inman procedures for KW tests. Critical values reported for the ANOVAs are the main effect degrees

of freedom, error degrees of freedom, F-ratio, and the associated p-value. For the KW tests the degrees of freedom and the H statistic are reported. All pair-wise p-values are also reported. Statistically significant differences are reported only for dose-related effects.

P1 Generation – Exposed Females (195-214 day old Breeders)

Blood was analyzed from female rats euthanized 5-8 weeks following a continuous 90-105 day exposure. Significant differences observed between dose groups compared to controls were an increase in RBC count ([F (3, 30) = 3.50, p = 0.027, ANOVA], C < M, p = 0.037) and hemoglobin concentrations ([F (3, 61) = 4.50, p = 0.027, ANOVA], C < M, p = 0.024) in female rats from the mid-dose group (Table 27).

P1 Generation – Exposed Males (163-172 day old Breeders)

Blood was analyzed from male rats euthanized 3-5 weeks after a continuous 90 day exposure. Significant differences observed between dose groups compared to controls were increases in hemoglobin concentrations ([F (3, 24) = 8.53, p = 0.001, ANOVA], C < L, p = 0.018, C < H, p = 0.001) in the low- and high-dose groups; increases in MCH ([H (3, 17.07) = 0.001, KW], C < H, p = 0.001) and MCH concentrations ([F (3, 24) = 10.69, p = 0.001, ANOVA], C < H, p = 0.001) in the high-dose group; and, increases in monocytes as a percentage of total WBCs ([H (3, 12.19) = 0.007, KW], C < L/M, p = 0.001, C < H, p = 0.006) in all of the dose groups (Table 28).

F1 Generation – Juvenile Females (PND 25-36)

Blood was analyzed from the juvenile F1 female offspring of exposed parents, which were euthanized following weaning and after completion of early development neurobehavioral tests. Significant differences observed between dose groups compared to controls were an increase in RBC count ([F (3, 30) = 4.96, p = 0.006, ANOVA], C < H, p = 0.013) and a decrease in RBC distribution width ([F (3, 30) = 12.26, p = 0.001, ANOVA], C > H, p = 0.001) in rats from the high-

dose group; and, increases in hematocrit ([H (3, 16.88) = 0.001, KW], C < M, p = 0.024, C < H, p = 0.046) and WBC counts ([H (3, 16.88) = 0.001, KW], C < M, p = 0.024, C < H, p = 0.046) in rats from the mid- and high-dose groups (Table 29).

F1 Generation – Juvenile Males (PND 25-36)

Blood was analyzed from the juvenile F1 male offspring of exposed parents, which were euthanized following weaning and after completion of early development neurobehavioral tests. Significant differences observed between dose groups compared to controls were an increase in RBC count ([H (3, 12.15) = 0.007, KW], C < H, p = 0.001) and a decrease in RBC distribution width ([F (3, 31) = 9.51, p = 0.001, ANOVA], C > H, p = 0.001) in rats from the high-dose group; and, an increase in hemoglobin concentrations ([H (3, 16.03) = 0.001, KW], C < H, p=0.001), hematocrit ([H (3, 15.29) = 0.002, KW], C < H, p = 0.001) and MCV ([F (3, 31) = 4.89, p = 0.007, ANOVA], C > H, p = 0.005) in rats from the high-dose group (Table 30). The WBC count was notably higher in the high-dose group compared to controls, but was not statistically significant.

F1 Generation – Unmated Adults (PND 94-105)

Blood was analyzed from the adult F1 female and F1 male offspring of exposed parents, which were exposed *in utero* to GD 19 and euthanized following the attainment of sexual maturity and after the completion of adult neurobehavioral tests. There were no significant dose-related hematological differences observed in the unmated adult offspring (Tables 31 and 32).

F1 Generation – Randomly Selected Breeders (PND 114-126)

Blood was analyzed from the adult F1 female and F1 male offspring of exposed parents, which were exposed *in utero* to GD 19 and euthanized following the weaning of F2 offspring and after completion of adult neurobehavioral tests. There were no significant dose-related hematological differences observed in the adult offspring selected as breeders (Tables 33 and 34).

F2 Generation (PND 23-28)

Blood was analyzed from the juvenile F2 offspring of parents exposed *in utero* to GD 19, which were euthanized following weaning and after completion of early development neurobehavioral tests. The only statistically significant dose-related difference observed between dose groups compared to controls was a decrease in WBC counts in female pups ([H (3, 8.56) = 0.036, KW], C > L, p = 0.023, C > M, p = 0.010, C > H, p = 0.006, Table 35) and male pups ([F (3, 27) = 4.91, p = 0.007, ANOVA], C > L, p = 0.027, C > M, p = 0.009, C > H, p = 0.054) across all dose groups (Table 36).

F2 Generation (Females: PND 93-101; Males: PND 86-99)

Blood was analyzed from the adult F2 offspring of parents exposed *in utero* to GD 19, which were euthanized following the attainment of sexual maturity and after completion of adult neurobehavioral tests. There were no significant dose-related hematological differences observed in adult F2 offspring (Tables 37 and 38).

Serum Chemistry

Differences in clinical chemistry values between dose groups were determined by a one-way ANOVA when data were found to be normal or by a non-parametric one-way Kruskal-Wallis (KW) test when data normality failed the Shapiro-Wilk test ($p < 0.05$). Pair-wise comparisons for the ANOVAs were performed using Tukey–Kramer procedures, or by using Conover-Inman procedures for the KW test. The critical values that are reported for the ANOVAs are the main effect degrees of freedom, error degrees of freedom, F-ratio, and the associated p-value. For KW tests the degrees of freedom and the H statistic are reported. Specific pair-wise p-values are also reported. Statistically significant differences are reported only for dose-related effects.

P1 Generation – Exposed Females (195-214 days old) and Males (163-172 days old)

Blood serum was analyzed from female breeders euthanized 5-8 weeks following a continuous 90-105 day exposure, and from male breeders sacrificed 3-5 weeks following a continuous 90-day exposure. The significant differences observed in the exposed female rats between dose groups compared to controls were decreases in alkaline phosphatase ([H (3, 12.05) = 0.007, KW], C > M, p = 0.034, C > H, p = 0.032) in rats from the mid- and high-dose groups, and an increase in potassium ion concentrations ([H (3, 8.04) = 0.045, KW], C < H, p = 0.049) in rats from the high-dose group (Table 39). There were no dose-related significant differences in serum chemistry observed in the exposed males between the dose groups in comparison to controls (Tables 40).

F1 Generation (PND 25-36)

Blood serum was analyzed from the juvenile F1 offspring of exposed parents, which were euthanized following weaning and after completion of early development neurobehavioral tests. The significant differences observed in female rats between dose groups compared to controls were increased levels of alanine aminotransferase ([F (3, 24) = 6.74, p = 0.002, ANOVA], C < H, p = 0.003); decreased glucose concentrations ([H (3, 13.88) = 0.003, KW], C > H, p = 0.003); and, decreased chloride ion ([F (3, 24) = 14.61, p = 0.001, ANOVA], C > H, p = 0.001) in the high-dose group (Table 41). The significant differences observed in male rats between dose groups compared to controls were increased concentrations of glucose ([F (3, 25) = 3.56, p = 0.029, ANOVA], C > H, p = 0.015) and triglycerides ([F (3, 25) = 4.37, p = 0.013, ANOVA], C > H, p = 0.010), and decreased blood urea nitrogen ([H (3, 8.39) = 0.039, KW], C > H, p = 0.038), in the high-dose group (Table 42).

F1 Generation – Unmated Adults (PND 94-105)

Blood serum was analyzed from the adult F1 female and F1 male offspring of exposed parents, who were exposed *in utero* to GD 19 and euthanized following the attainment of sexual maturity and after completion of adult neurobehavioral tests. There were no significant dose-related hematological differences observed in the unmated adult female offspring (Tables 43).

The only statistically significant dose-related difference observed in male rats between dose groups compared to controls was a decrease in triglyceride levels ([F (3, 21) = 5.98, p = 0.004, ANOVA], C > M, p = 0.003, C > H, p = 0.027) in the mid- and high-dose groups (Table 44).

F1 Generation – Randomly Selected Breeders (PND 114-126)

Blood serum was analyzed from the adult F1 female and F1 male offspring of exposed parents, who were exposed *in utero* to GD 19 and euthanized following the weaning of F2 offspring and after completion of adult neurobehavioral tests. Statistically significant dose-related differences observed in female rats among the dose groups in comparison to controls were increased concentrations of creatinine ([H (3, 11.47) = 0.009, KW], C < M, p = 0.003, C < H, p = 0.002) in the mid- and high-dose groups and blood urea nitrogen ([H (3, 18.01) = 0.001, KW], C < L/M/H, p = 0.001) in all of the dose groups (Table 45). The statistically significant differences observed in male rats between dose groups compared to controls were increased concentrations of total protein ([F (3, 22) = 8.80, p = 0.001, ANOVA], C < L, p = 0.012, C < H, p = 0.001), blood urea nitrogen ([F (3, 22) = 14.28, p = 0.001, ANOVA], C < L, p = 0.001, C < H, p = 0.033), and glucose ([F (3, 22) = 5.30, p = 0.007, ANOVA], C < L, p = 0.034, C < H, p = 0.007) in the mid- and high-dose groups (Table 46).

F2 Generation (PND 23-28)

Blood serum was analyzed from the juvenile F2 offspring of parents exposed *in utero* to GD 19, who were euthanized following weaning and completion of early development neurobehavioral

tests. The only statistically significant difference observed in female rats between dose groups compared to controls was increased alkaline phosphatase ([H (3, 9.36) = 0.026, KW], C < L, p =0.005, C < M, p = 0.006, C < H, p = 0.090) in the low- and mid-dose groups (Table 47). The statistically significant differences observed in male rats between dose groups compared to controls were increased levels of total protein ([H (3, 14.73) = 0.002, KW], C < H, p = 0.001), albumin ([H (3, 16.43) = 0.001, KW], C < H, p = 0.001), creatinine ([H (3, 15.30) = 0.002, KW], C < H, p = 0.001), total bilirubin ([H (3, 9.28) = 0.026, KW], C < H, p = 0.006), triglycerides ([H (3, 9.87) = 0.020, KW], C < H, p = 0.006) and sodium ion ([H (3, 10.20) = 0.017, KW], C < H, p = 0.003) in high-dose group (Table 48).

F2 Generation (Females: PND 93-101; Males: PND 86-99)

Blood serum was analyzed from the adult F2 offspring of parents exposed *in utero* to GD 19, who were euthanized following attainment of sexual maturity and after completion of adult neurobehavioral tests. There were no significant dose-related differences in serum chemistry observed in adult F2 offspring (Tables 49 and 50).

Histopathology

Differences between the proportion of rats exhibiting specific pathologies for each dose group were compared using the Pearson Chi-square test (X^2, α=0.05) and all post-hoc comparisons were performed using Fisher's exact test for pair-wise comparisons. All proportions were based on incidence data only, and not the severity of a histological finding. The critical values that are reported for X^2 tests are the degrees of freedom, sample size, and the X^2 statistic. Pair-wise p-values are also reported, as well as p-values ≤ 0.05 for certain critical study measures. Only statistically significant values are presented in complete (APA) format. Tissues from control and dose groups were prepared for microscopic examination following necropsy. A detailed report of histopathological findings is included as Appendix A.

P1 Generation – Exposed Females (195-214 day old Breeders)

There were no statistically significant incidences of pathological findings identified in the adrenal glands, brain, heart, kidneys, liver, mammary gland, ovaries, oviducts, pancreas, pituitary gland, spleen, uterus or uterine horns taken from female rats euthanized 5-8 weeks after a continuous 90-105 day exposure (Table 51). Idiopathic findings were not considered noteworthy or dose-related. Also, the results of a macroscopic examination of uteruses from the exposed dams indicate that the numerical differences between identified uterine implantation sites and actual litter size were not statistically significant for dams from the high-dose group in comparison to controls (Table 14).

P1 Generation – Exposed Males (163-172 day old Breeders)

There were no statistically significant incidences of pathological findings identified in the adrenal glands, brain, epididymides, kidneys, liver, pancreas, pituitary gland, spleen or testes taken from male rats euthanized 3-5 weeks after a continuous 90-day exposure. However, there were significant proportional differences among the groups for male rats that were observed to have progressive cardiomyopathy (X^2 = 15.71, 3, p = 0.001, Table 52), with the proportions of male rats from the mid- and high-dose groups presenting fewer incidents compared to controls (p = 0.007). In addition, the results of a testicular spermatogenesis staging evaluation of exposed male rats indicated no significant microscopic lesions in the testes evaluated (Appendix A).

F1 Generation (PND 25-36)

There were no statistically significant incidences of pathological findings identified in the adrenal glands, brain, epididymides, heart, kidneys, liver, mammary gland, ovaries, oviducts, pancreas, pituitary gland, spleen, testes, uterus or uterine horns taken from juvenile, F1 female (Table 53) and F1 male (Table 54) offspring of exposed parents, which were euthanized after weaning and the completion of early development neurobehavioral tests.

F1 Generation (PND 94-105)

There were no statistically significant incidences of pathological findings identified in the adrenal glands, brain, epididymides, heart, mammary gland, ovaries, oviducts, pancreas, pituitary gland, spleen, testes, uterus or uterine horns taken from the adult, first generation female (Table 55) and male (Table 56) offspring of exposed parents, which were not selected for breeding. There were also no statistically significant pathological observations identified in the kidneys or livers of female rats. However, there were significant proportional differences identified among the male groups relating to lymphohistiocytic infiltration in the kidneys (X^2 = 9.55, 3, p = 0.023, Table 56) and in the liver (X^2 = 10.26, 3, p = 0.016, Table 56). Proportional differences among males in the dose groups exhibiting this finding in the kidney did not significantly differ from controls; however, the proportional differences among males in the dose groups exhibiting this finding in the liver were significantly reduced, when compared to controls (p = 0.026). These rats were euthanized after the completion of adult neurobehavioral tests.

F1 Generation – Randomly Selected Breeders (PND 114-126)

There were no statistically significant incidences of pathological findings identified in the adrenal glands, brain, epididymides, kidneys, mammary glands, ovaries, oviducts, pancreas, pituitary gland, spleen, testes, uterus or uterine horns taken from the adult, F1 female (Table 57) and F1 male (Table 58) offspring of exposed parents, which were randomly selected as breeders. Additionally, there were no significant pathological observations identified in the hearts of successfully mated females or the livers of successfully mated males. Significant proportional differences were identified among the groups for dams observed with liver lesions (X^2 = 10.19, 3, p = 0.017) and lymphohistiocytic infiltration of the liver (X^2 = 9.93, 3, p = 0.019, Table 57); however, the proportions of dams from the dose groups exhibiting these findings did not significantly differ from controls. Additionally, the proportions of sires identified with minimal progressive cardiomyopathy varied among groups (X^2 = 8.14, 3, p = 0.043, Table 58), with the

proportion of male rats exhibiting this finding in the high-dose group being significantly elevated in comparison to controls (p = 0.041). The rats were euthanized after weaning F2 pups and completing adult neurobehavioral testing.

F2 Generation (PND 23-28)

There were no statistically significant incidences of pathological findings identified in the adrenal glands, brain, epididymides, heart, liver, mammary glands, ovaries, oviducts, pancreas, pituitary gland, spleen, testes, uterus or uterine horns taken from the unexposed, juvenile, F2 female (Table 59) and F2 male (Table 60) offspring of parents exposed *in utero* to GD 19. Additionally, there were no pathological observations identified in the kidneys of female offspring; however, significant proportional differences were identified in male offspring observed to have kidney lesions (X^2 = 7.88, 3, p = 0.048, Table 60). Pair-wise comparison of proportional differences of male rats exhibiting these findings from the dose groups did not significantly differ from controls. Rats were euthanized after weaning and completing early development neurobehavioral tests.

F2 Generation (Females: PND 93-101; Males: PND 86-99)

There were no statistically significant incidences of pathological findings identified in the adrenal glands, brain, epididymides, heart, kidneys, liver, mammary glands, ovaries, oviducts, pituitary gland, spleen, testes or uterus taken from the unexposed, adult, F2 female (Table 61) and F2 male (Table 62) offspring of parents exposed *in utero* to GD 19. There were also no significant pathological observations identified in the pancreas of the adult females; however, there were proportional differences in the female rats observed to have dilation of their uterine horns (any severity) between groups (X^2 = 9.03, 3, p = 0.029, Table 61), with statistically lower proportions of dilation in the dose groups in comparison to controls. There were also differences in the proportions of adult male rats observed to have chronic inflammation of the pancreatic acinus (any severity) among groups (X^2 = 7.88, 3, p = 0.048, Table 62); however, the proportions of

male rats in the dose groups exhibiting these findings did not significantly differ compared to controls. Rats were euthanized following attainment of sexual maturity and after completion of adult neurobehavioral tests.

Neurobehavioral Assessments

Differences in neurobehavioral measures between dose groups were determined by a two-way ANOVA when data were found to be normal, or by a non-parametric one-way Kruskal-Wallis (KW) test when data normality failed the Shapiro-Wilk test ($p<0.05$). Pair-wise comparisons for the ANOVAs were performed using Tukey–Kramer procedures, or by using Conover-Inman procedures for KW tests. Critical values reported for the ANOVAs are the main effect degrees of freedom, error degrees of freedom, F-ratio, and the associated p-value. For the KW tests the degrees of freedom and the H statistic are reported. All pair-wise p-values are also reported. All exposed parents, a subset of their offspring (F1), and a subset of their offspring (F2) were evaluated for neurobehavioral effects using a small battery of neurobehavioral tests selected from the neurotoxicity assessment battery (NTAB) developed in our laboratory. No significant dose related effects were found for the exposed parents or either generation of adult offspring. A few minor dose-related effects were observed in F1 and F2 offspring for early developmental tests. The neurobehavioral results are summarized in Tables 63-67.

P1 Generation

Motor Activity

There were no significant differences detected between the dose groups and controls for the exposed male parents (P1 rats) for any of the motor activity measurements, including the total distance traveled, activity time, average speed, total rears, and percentage of time in center vs. perimeter (Table 63; only total activity times in seconds are shown in the table). However, the exposed female parent (P1) control group was inadvertently removed from the study prior to

completing motor activity testing; therefore, there were no female controls for comparison during the analyses of the tests. Nevertheless, existing evidence suggests the absence of significant dose-related effects in exposed female rats, since there were no significant differences detected in motor activity among the female dose groups (Table 63), and no effects were discovered in exposed female parents from the 28 day (Phase II) study, which were evaluated by the same tests (NAMRU-D-12-03).

Water Maze Learning and Memory

There were no significant differences detected between the dose groups and controls for the exposed male parents (P1 rats) during the 5-day learning phase for water maze navigation or during the 24-hour probe trial to test memory function (Table 63; Figure 3). However, since the exposed female parent (P1) control group was inadvertently removed from the study prior to completing water maze testing, there were no female controls for comparison during the data analyses of the tests. Nevertheless, existing evidence suggests the absence of significant dose-related effects in exposed female rats, since there were no significant differences detected among the female dose groups during the 5-day learning phase for water maze navigation, with exception that females from the high-dose group were slower than females from the mid- and low-dose groups on learning day 3, which effect was not persistent across the learning curve (Figure 4). In addition, there were no significant differences detected among the dose groups during the 24-hour probe trial to test the memory function of the female P1 rats (Table 63). Moreover, no effects were discovered in exposed female parents from the 28 day (Phase II) study, which were evaluated by the same tests (NAMRU-D-12-03).

Maternal Retrieval

There were no significant differences detected among groups for the latency of exposed (P1) dams to retrieve three random pups (Table 64).

F1 Generation (Pup Tests)

Righting Reflex

A natural gender effect, independent of exposure, was previously identified for righting reflex in Phase 2 (NAMRU-D-12-03). Therefore, male and female pups were analyzed separately during Phase 3 to correct for gender differences. The only statistically significant effect detected was a slightly increased latency in low-dose group male offspring to right themselves in comparison to controls ([F (3, 29) = 3.67], C < L, p = 0.009, ANOVA, Table 64). This effect was not observed in male offspring from the mid- or high-dose groups.

Separation Distress

There were no significant dose-related effects detected in ultrasonic distress vocalizations ("calls to mom") for either male or female offspring (Table 64).

F1 Generation (Adult Tests)

Maternal Retrieval

There were no significant dose-related effects detected in the latency of F1 dams to retrieve 3 random pups (Table 64).

Motor Activity

There were no significant differences detected between dose groups and controls for motor activity in adult F1 generation males or females (Table 65; only total activity times in seconds are shown in the table). Measurements of total distance, activity time, average speed, total rears and percentage of time in center vs. perimeter during spontaneous activity assessments were similar between groups.

Water Maze Learning and Memory

There were no significant differences detected between dose groups and controls during the 5-day learning phase for water maze navigation, with the exception that dams from the mid-dose group required significantly more time to find the platform than dams from the low-dose group on learning day 5 ([F (3, 28) = 3.68], M>L, p = 0.024, ANOVA, Table 65). Figure 5 (sires) and Figure 6 (dams) graphically compare the learning performance of first generation offspring over the 5-day training cycle. Significant dose-related effects were not detected between groups during the 24-hour probe trial to test the memory function of P1 rats (Table 65).

F2 Generation (Pup Tests)

Righting Reflex

There were no statistically significant dose-related effects detected in second generation male and female pups analyzed independently for righting latency (Table 66).

Separation Distress

A single significant dose-related effect was detected for ultrasonic distress vocalizations in the F2 offspring, as female pups from the control group vocalized significantly more than females from the dose groups ([F (3, 123) = 4.73], C > L/M/H, p = 0.004, ANOVA, Table 66). Male pups from the control group also vocalized more than males from the dose groups, but the effect was not statistically significant.

F2 Generation (Adult Tests)

Motor Activity

There were no significant differences detected between the dose groups and controls for motor activity in the adult F2 rats. Measures of total distance, activity time, average speed, total rears and percentage of time in center vs. perimeter were similar between groups.

Water maze Learning and Memory

There were no significant differences detected between dose groups and controls during the 5-day learning phase for water maze navigation, with the exception that males from the low-dose group found the platform significantly faster than controls on learning day 2 ([F (3, 28) = 3.95], C > L, p = 0.018, ANOVA, Table 67). Figure 7 (males) and Figure 8 (females) graphically compare the learning performance of first generation offspring over the 5-day training cycle. Significant dose-related effects were not detected between groups during the 24-hour probe trial to test the memory function of F2 rats (Table 67).

Discussion

The purpose of this study was to evaluate potentially adverse submarine atmosphere exposure effects on female and male crew members, with a special focus upon reproductive system risks, using a rat model. All the evaluated measures of reproduction were within the normal historical ranges for the tested strain of rat after 90 days of exposure and adverse pregnancy outcomes were statistically non-significant in comparison to controls. The mating of F1 offspring, which were exposed *in utero* to GD 19, also indicated no abnormal or adverse reproductive outcomes. In fact, the only reproductive measure that showed a statistically significant difference was that the gestation index for F1 dams indicated that pregnant dams from the mid and high-dose groups had a higher proportion of live litters in comparison to controls. Evaluated measures of development indicated no statistically significant differences among F1 and F2 offspring from the dose groups compared to controls pertaining to growth, sensory perception and/or viability. Among the several measures of general health that were evaluated, five statistically significant physiological differences were observed. First, the average weight gains in exposed male rats from the mid and high-dose groups were significantly reduced in comparison to average weight gains in rats from the control or low-dose groups. The biological significance of these reduced weight gains was marginal for males from the high-dose group (9.7% reduction in comparison to

controls), and was minimal for males from the mid-dose group (4.3% reduction in comparison to controls), based upon U.S. EPA's clinical criteria for adverse health effects based on weight loss (loss ≥ 10%). There were no similar reductions in weight gain observed in the exposed females.

Second, statistically significant increases in RBC count, hemoglobin concentration and percent hematocrit were common in the exposed rats and juvenile F1 offspring from mid and high-dose groups in comparison to controls. The up-regulation of erythropoiesis is an established effect of long-term hypoxia and is considered to be an adaptive response, not an adverse effect (Quinlan et al, 1998). Since blood was drawn from the animals at necropsy, which was 3-5 weeks post-exposure for P1 males and juvenile F1 offspring exposed *in utero*, and 5-8 weeks post-exposure for P1 females, it is reasonable to assume that short-lived exposure effects would have resolved before the blood analysis, indicating that the effect is persistent. However, these parameters did not remain statistically significant in F1 adults and F2 offspring.

Differences in WBC count were statistically significant in juvenile females from both generations. However, while the WBC count increased in the F1 pups from the dose groups in comparison to controls, the WBC count decreased in the F2 pups from the dose groups compared to controls. The etiology of these opposed results is not known and may simply reflect normal variability in the development of the immune systems in these rat pups, since none of the adult populations showed similar effects. The physiological significance of the differences is considered minimal, since all hematology and serum chemistry analyses remained within, or near, the normal ranges established for this strain of rat respective of dose groups, age specific groups, and generations; and, (2) histopathological examination of livers and spleens in the rats failed to confirm clinical significance to the hematological findings, as did a lack of significant differences in liver enzyme levels in the blood serum.

Third, kidney weights measured in exposed male rats from the dose groups were significantly reduced in comparison to controls. However, since histopathological analysis found a higher incidence of nephropathy and a higher number of kidney lesions in rats from the control group compared to the dose groups, adverse, dose-related kidney effects are not indicated by these kidney weight differences. As previously noted in this study, early stage chronic progressive nephropathy and other degenerative kidney processes are commonly found in this strain of rat (Turnbull et al., 1985) and were identified in kidney tissues across groups. The most common cause of the disease is protein overload, with the proximal convoluted tubule being the most common site for this lesion. The clinical severity of the lesions identified was minimal, with no evidence of ischemia or hypoxia present in examined sections. Moreover, the frequency of the lesions was associated with increasing age. All kidney findings were considered to be unrelated to exposure, since there were no statistically significant increases in the percentage incidence of any renal disease process in rats from the dose groups in comparison to controls for the three generations examined.

Fourth, the average liver weight in first generation dams was significantly increased in females from the dose groups in comparison to controls. However, since the histopathological analysis detected a higher incidence of lymphohistiocytic infiltration and inflammation in dams from the control group in comparison to the dose groups, adverse dose-related effects are not indicated by these liver weight differences. Small clusters of infiltrates in the hepatic sinusoids of the liver were observed in nearly all of the groups, including controls. The background incidence of this minimal finding is naturally high for this particular strain of rat, and all statistically significant differences found were higher in the controls than in the dose groups. In addition, clinical chemistry analysis of the blood serum indicated that the enzyme used as the biomarker for liver damage (AST) was not elevated in any dose groups in comparison to controls. Therefore, all liver disease processes are considered to be spontaneous and incidental to these exposures.

Fifth, a statistically significant difference (increase) in cardiomyopathy (of minimal severity) was discovered in adult F1 sires from the high-dose group in comparison to controls; however, since adult F1 males not selected for mating showed the highest incidence of this pathology within the control group, this adverse effect is not considered to be dose-related.

None of these physiological differences were sufficient to demonstrate a clear adverse effect. To quote the final report (Appendix A) from Seventh Wave Histology Laboratory,

> "Across the three generations examined and the different ages, microscopic findings in rats exposed to the test atmospheres or rats descended from those exposed to the test atmospheres were not considered test atmosphere related because they either were singular in occurrence, were present at a comparable incidence in the corresponding control rats, did not show a relationship to increasing CO and CO_2 concentration, and/or represent common, spontaneous, "background" findings in rats of this strain and the respective ages examined."

Neurological performance testing revealed no associated effects between exposure status and any change in emotionality, activity levels, exploratory behaviors, and/or higher-level cognitive functions (learning and memory) in tests conducted on the exposed parents or adult offspring.

A single significant neurobehavioral difference was discovered in the F1 generation for the early developmental motor coordination task of righting to all four paws, where male pups from the low-dose group were significantly slower to right themselves in comparison to controls. Since pups from the mid and high-dose groups showed very similar reaction times to the controls, and no similar effect was observed in the F2 pups, the results are not considered to be dose-related.

A single significant neurobehavioral difference was also discovered in the F2 generation, where female pups from the control group were more vocal than were the pups from the dose groups. Since similar test results were not found in the F1 pups, which had a control group vocalization rate similar to rates measured in the F1 and F2 dose groups, it is likely that the F2 control group contains an anomalous set of data, rather than vocalization rate being a true dose-related effect. The difference may be related to a scheduling error causing the entire control group to be tested on PND 7 and the dose groups to be tested on PND 8. An automated system that is capable of detecting multiple fast vocalizations with greater sensitivity was used during the Phase 3 testing and suggests that age may be a more sensitive factor than previously assumed.

In conclusion, 90-day exposures to the three submarine test atmospheres (CO, CO_2, and O_2) did not affect the ability of rats to reproduce and did not result in any significant developmental deficits in their offspring. The physiological effects considered to be dose-related were reduced weight gains of marginal biological significance and the adaptive up-regulation of erythropoiesis, both effects being most notable in male rats from the high-dose group. Evidence of general toxicity, endocrine disruption, and abnormal behavioral effects was absent.

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Table 1: Parameters Measured for P1, F1 and F2 Generations

Generation	Parameter
P1	Weight Gain While Under Exposure
P1	Hormone and Vitamin D Levels
P1	Blood Gases (Carboxyhemoglobin)
P1; F1	Estrous Cycle
P1; F1	Mating Success
P1; F1	Delivery Success
P1; F1	Gestation Length
P1; F1	F1 PND 0 Litter Size (Total Pups and Live Pups)
P1; F1	F1 PND 0 Litter Weight (Male and Female)
P1; F1	F1 PND 0 Sex Ratio
P1; F1	Maternal Care of Young
F1; F2	Gross Malformations PND 1-4
F1; F2	PND 1-21 Weight Gain
F1; F2	PND 1-21 Survival Rates
F1; F2	Neurobehavioral Assessments PND 3-8
F1; F2	PND 4 Survival
F1; F2	Days to Eyes and Ears Open
P1; F1; F2	Neurobehavioral Assessments PND 51+
P1; F1; F2	Hematology
P1; F1; F2	Serum Chemistries
P1; F1; F2	Post-Necropsy Tissue Weights
P1; F1; F2	Histopathology of Target Tissues

Table 2: Neurobehavioral Testing Schedule.

Test	Number of Animals	Day
Maternal Retrieval (P1 Dams)	32 Exposed Dams	PND 2 or 3
Maternal Retrieval (F1 Dams)	32 Dams (exposed *in utero*)	PND 2 or 3
1st Litter Cull – PND4 (Retained Pups: 4 Males + 4 Females per Litter)		
Righting Reflex (F1 Offspring)	256 Pups (equal sex ratio) (32 litters exposed *in utero*)	PND 4 or 5
Righting Reflex (F2 Offspring)	256 Pups (equal sex ratio) (32 unexposed litters)	PND 4 or 5
Separation Distress (F1 Offspring)	256 Pups (equal sex ratio) (32 litters exposed *in utero*)	PND 7 or 8
Separation Distress (F2 Offspring)	256 Pups (equal sex ratio) (32 unexposed litters)	PND 7 or 8
2nd Litter Cull (Weaning) – PND 19-23 (Retained Pups: 1 Male + 1 Female per Litter)		
Motor Activity and Water Maze (P1 – Parents)	32 Exposed Sires 24 Exposed Dams*	19-23 Days Post Exposure
Motor Activity and Water Maze (F1 Offspring)	32 Sires (exposed *in utero*) 32 Dams (exposed *in utero*)	PND 50-80
Motor Activity and Water Maze (F2 Offspring)	32 Unexposed Males 32 Unexposed Females	PND 50-80

* The female parent control group was inadvertently removed from the study prior to motor activity and water maze testing.

Table 3: Summary of inhalation exposure data: whole body exposure system (Environmental parameters from 90-day sub-chronic exposure period)

			Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Temp	(Deg C)	Mean	22.5	23.6	23.9	23.1
		St Dev	1.0	1.6	1.1	1.3
		Min	20.8	19.8	19.7	19.2
		Max	29.5	29.3	25.6	24.7
		Count	3 [a]	97	95	99
Humidity	(%)	Mean	50	61	63	60
		St Dev	2	9	10	7
		Min	19	34	36	43
		Max	67	80	82	82
		Count	3 [a]	97	95	99
Supply Air Flow Rate	(L/min)	Mean	408	183	171	162
		St Dev	3	2	2	2
		Min	382	175	169	160
		Max	441	193	182	175
		Count	3 [a]	97	95	99
Carbon Monoxide Concentration	(ppm)	Mean	0 .42	5.0	13.9	89.9
		St Dev	0 .02	0.5	0.5	2.8
		Min	0.37	3.4	12.1	79.4
		Max	0 .48	7.7	15.0	95.0
		Count	92	97	95	99
Carbon Dioxide Concentration	(%)	Mean	0.13	0.41	1.19	2.47
		St Dev	0.02	0.02	0.05	0.08
		Min	0.06	0.33	1.05	2.11
		Max	0.15	0.47	1.31	2.66
		Count	92	97	95	99
Oxygen Concentration	(%)	Mean	20.6	17.1	16.1	15.0
		St Dev	0.6	0.2	0.2	0.3
		Min	15.5	16.4	15.3	14.0
		Max	21.3	17.8	16.9	16.1
		Count	92	97	95	87 [b]
Static Pressure	(in H_2O)	Mean	0.08	-0.09	-0.09	-0.08
		St Dev	0.04	0.05	0.03	0.04
		Min	0.04	-0.19	-0.16	-0.19
		Max	0.11	0.05	-0.03	0.03
		Count	3 [a]	97	95	99

[a] Represents the mean and standard deviation of the three individual means for the three control chambers. The minimum and maximum are the lowest and highest daily means from all 276 values.

[b] Oxygen monitor malfunction resulting in missing or suspect data for 12 days.

Table 4: Summary of inhalation exposure data: whole body exposure system (Environmental parameters from 90-minute blood gas exposure period)

			Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Temp	(Deg C)	Mean	22.3	21.0	21.5	21.9
		St Dev	0.5	0.0	0.3	0.1
		Min	21.1	20.9	21.0	21.8
		Max	23.4	21.0	22.0	22.2
		Count	1375	1134	1086	1147
Humidity	(%)	Mean	29.4	24.9	31.6	28.2
		St Dev	1.0	0.0	0.3	0.8
		Min	27.4	24.9	30.8	26.6
		Max	32.2	24.9	32.2	29.3
		Count	1375	1134	1086	1147
Supply Air Flow Rate	(L/min)	Mean	227	183	175	163
		St Dev	1	0	1	0
		Min	226	182	172	162
		Max	228	184	176	163
		Count	1375	1134	1086	1147
Carbon Monoxide Concentration	(ppm)	Mean	0 .21	5.3	13.8	91.9
		St Dev	0 .04	0.9	0.2	0.3
		Min	0.00	2.7	13.1	91.4
		Max	1.11	6.1	14.3	93.0
		Count	1375	1134	1086	1147
Carbon Dioxide Concentration	(%)	Mean	0.13	0.42	1.2	2.5
		St Dev	0.01	0.00	0.0	0.0
		Min	0.12	0.41	1.2	2.5
		Max	0.14	0.42	1.2	2.6
		Count	1375	1134	1086	1147
Oxygen Concentration	(%)	Mean	21.1	17.1	16.1	15.1
		St Dev	0.0	0.0	0.0	0.0
		Min	21.1	17.1	16.1	15.0
		Max	21.2	17.1	16.1	15.1
		Count	1375	1134	1086	1147
Static Pressure	(in H_2O)	Mean	1.0	1.0	1.0	1.1
		St Dev	0.0	0.0	0.0	0.0
		Min	1.0	1.0	1.0	1.0
		Max	1.1	1.1	1.1	1.1
		Count	1375	1134	1086	1147

Table 5: Summary of inhalation exposure data: nose only exposure system (Environmental parameters from 90-minute blood gas exposure period)

			Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Temp	(Deg C)	Mean	22.1	22.7	21.6	21.9
		St Dev	0.6	0.3	0.2	0.1
		Min	21.4	22.3	21.5	21.8
		Max	22.8	23.1	21.8	21.9
		Count	4 [a]	5 [a]	4 [a]	4 [a]
Humidity	(%)	Mean	30.5	19.8	41.0	43.5
		St Dev	0.6	0.4	0.0	1.3
		Min	30.0	19.0	41.0	42.0
		Max	31.0	20.0	41.0	45.0
		Count	4 [a]	5 [a]	4 [a]	4 [a]
Supply Air Flow Rate	(L/min)	Mean	26.4	27.0	26.4	26.4
		St Dev	0.0	0.0	0.0	0.0
		Min	26.4	27.0	26.4	26.4
		Max	26.4	27.0	26.4	26.4
		Count	4 [a]	5 [a]	4 [a]	4 [a]
Carbon Monoxide Concentration	(ppm)	Mean	0 .31	5.2	14.2	92.6
		St Dev	0 .29	0.7	0.2	0.3
		Min	0.00	3.0	13.1	92.0
		Max	1.03	5.8	14.6	94.0
		Count	1375	1134	1086	1147
Carbon Dioxide Concentration	(%)	Mean	0.13	0.41	1.2	2.5
		St Dev	0.00	0.00	0.0	0.0
		Min	0.13	0.41	1.2	2.5
		Max	0.14	0.41	1.2	2.5
		Count	1375	1134	1086	1147
Oxygen Concentration	(%)	Mean	21.0	17.1	16.1	15.0
		St Dev	0.0	0.0	0.0	0.0
		Min	21.0	17.1	16.1	15.0
		Max	21.1	17.1	16.1	15.1
		Count	1375	1134	1086	1147
Static Pressure	(in H_2O)	Mean	-0.07	-0.07	-0.08	-0.05
		St Dev	0.02	0.01	0.00	0.02
		Min	-0.10	-0.08	-0.08	-0.06
		Max	-0.05	-0.06	-0.08	-0.03
		Count	4 [a]	5 [a]	4 [a]	4 [a]

[a] Data were recorded into the study notebook during the exposure.

Table 6: Inhalation exposure summary data: test chemical flow rates (Environmental parameters from 90-day sub-chronic exposure period)

			Group 2 Low-Dose	**Group 3** Mid-Dose	**Group 4** High-Dose
Carbon Monoxide Flow Rate	(mL/min)	Mean	1.0	2.6	14.7
		SD	0.1	0.1	0.8
		Min	0.8	2.3	12.1
		Max	1.2	2.8	16.0
		Count	96	95	98
Carbon Dioxide Flow Rate	(L/min)	Mean	0.23	1.99	5.10
		SD	0.13	0.14	0.26
		Min	0.13	1.63	4.40
		Max	0.81	2.54	5.82
		Count	96	95	98
Nitrogen Flow Rate	(L/min)	Mean	32.7	42.9	51.2
		SD	1.7	1.9	2.6
		Min	22.3	29.5	34.5
		Max	38.8	46.6	56.0
		Count	96	95	98

Table 7: Inhalation exposure summary data: test chemical flow rates (Environmental parameters from 90-minute blood gas exposure period)

			Group 1 Control	**Group 2** Low-Dose	**Group 3** Mid-Dose	**Group 4** High-Dose
Carbon Monoxide Flow Rate	(mL/min)	Mean	NA	1.2	2.9	14.3
		St Dev	NA	0.1	0.0	0.0
		Min	NA	1.1	2.9	14.3
		Max	NA	1.5	3.0	14.5
		Count	NA	1134	1086	1147
Carbon Dioxide Flow Rate	(L/min)	Mean	0.15	0.81	2.9	6.1
		St Dev	0.00	0.00	0.0	0.0
		Min	0.14	0.81	2.9	6.1
		Max	0.15	0.82	3.0	6.1
		Count	1375	1134	1086	1147
Nitrogen Flow Rate	(L/min)	Mean	NA	39.2	47.5	54.9
		St Dev	NA	0.0	0.0	0.0
		Min	NA	39.2	47.4	54.9
		Max	NA	39.2	47.5	55.0
		Count	NA	1134	1086	1147

Table 8: Weight Change Data (±SD) in female rats during 8 weeks of pre-mating exposure (weight differences between 10 and 18 week old rats); n=(x).

Endpoint	**Group 1** Control	**Group 2** Low-Dose	**Group 3** Mid-Dose	**Group 4** High-Dose
Initial Weight (grams)	260.3 ± 18.3 (32)	250.2 ± 19.0 (32)	246.4 ± 15.3 (32)	254.1 ± 18.9 (32)
Final Weight (grams)	317.0 ± 33.1 (31)	305.8 ± 33.3 (32)	308.4 ± 23.3 (31)	305.4 ± 24.2 (32)
Weight Change (grams)	56.7 ± 19.6	55.6 ± 16.8	61.8 ± 13.7	51.3 ± 18.4
% Weight Change	Δ = + 21.8 %	Δ = + 22.2 %	Δ = + 25.1 %	Δ = + 20.2 %

Table 9: Weight Change Data (±SD) in male rats during 8 weeks of pre-mating exposure (weight differences between 10 and 18 week old rats); n=(x).

Endpoint	**Group 1** Control	**Group 2** Low-Dose	**Group 3** Mid-Dose	**Group 4** High-Dose
Initial Weight (grams)	409.9 ± 26.3 (32)	378.7 ± 35.4 (32)	404.3 ± 30.2 (32)	412.1 ± 35.4 (32)
Final Weight (grams)	578.1 ± 38.4 (30)	551.2 ± 48.9 (32)	550.6 ± 47.1 (32)	539.5 ± 54.2 (32)
Weight Change (grams)	166.2 ± 23.8	172.5 ± 27.5	**146.3Ŧ ± 30.2**	**127.4Ŧ ± 35.7**
% Weight Change	Δ = + 40.5 %	Δ = + 45.5 %	Δ = + 36.2 %	Δ = + 30.8 %

Ŧ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

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Table 10: Summary of selected serum hormone levels (±SD) from rats after 69 Days exposure and prior to mating.

Endpoint	Exposed Males		Exposed Females	
	Control	High-Dose	Control	High-Dose
Dehydroxepiandrosterone (DHEA)[1] (ng/mL)	43.1 ± 7.0 (6)	41.9 ± 7.4 (8)	74.6 ± 24.5 (6)	82.3 ± 47.9 (6)
Estradiol[1] (pg/mL)	48.9 ± 8.8 (6)	42.9 ± 14.0 (8)	64.6 ± 65.4 (6)	77.3 ± 104.6 (5)
Follicle Stimulating Hormone (FSH)[2] (ng/mL)	<0.20 (6)	0.50 ± 0.84 (8)	0.35 ± 0.34 (5)	2.72 ± 3.22 (6)
Luteinizing Hormone (LH)[3] (ng/mL)	1.83 ± 1.82 (6)	4.04 ± 3.35 (8)	0.90 ± 1.30 (5)	**9.78Ŧ ± 5.32** (6)
Progesterone[1] (ng/mL)	1.79 ± 0.32 (6)	1.41 ± 0.46 (8)	17.5 ± 14.2 (6)	15.6 ± 20.9 (6)
Testosterone[1] (ng/mL)	678 ± 1073 (5)	858 ± 901 (8)	21 ± 10 (5)	11 ± 5 (6)
Vitamin D (as 1,25-Dihydroxyvitamin D3)[4] (ng/mL)	1.29 ± 0.15 (6)	1.33 ± 0.21 (8)	2.71 ± 0.34 (5)	2.81 ± 0.34 (6)

Ŧ Student t-tests indicated a statistically significant difference between the high-dose group and controls for increased serum concentrations of luteinizing hormone.

1 – Meso Scale Discovery MULTI-SPOT Assay System Custom Steroid Hormone Panel (human/mouse/rat)

2 – Shibayagi Co. Ltd. Rat FSH ELISA Kit

3 – Shibayagi Co. Ltd. Rat LH ELISA Kit

4 – Cusabio Rat 1, 25-Dihydroxyvitamin D3 ELISA Kit

Table 11: Summary of estrus cycle monitoring data

Estrus Cycle Phase	Exposed Parents[1]				F1 Offspring[2]			
	Controls n = 8[a]; 72[b]	**Low-Dose** n = 8[a]; 79[b]	**Mid-Dose** n = 8[a]; 80[b]	**High-Dose** n = 8[a]; 77[b]	**Controls** n = 8[a]; 108[b]	**Low-Dose** n = 8[a]; 102[b]	**Mid-Dose** n = 8[a]; 105[b]	**High-Dose** n = 8[a]; 107[b]
Proestrus	11 (15.3%)	6 (7.6%)	17 (21.3%)	5 (6.5%)	18 (16.7%)	17 (16.7%)	14 (13.3%)	7 (6.5%)
Estrus	18 (25.0%)	25 (31.6%)	13 (16.2%)	21 (27.3%)	29 (26.8%)	33 (32.3%)	32 (30.5%)	43 (40.2%)
Metestrus and Diestrus	43 (59.7%)	48 (60.8%)	50 (62.5%)	51 (66.2%)	61 (56.5%)	52 (51.0%)	59 (56.2%)	57 (53.3%)

[1] Data were collected for 4 or 2 consecutive days, interrupted by a 2-day break, collected for an additional 5 days followed by a 2 day break and the collected for an additional 2 to 4 consecutive days.

[2] Data were collected over 14 consecutive days.

[a] value indicates the number of rats per group

[b] value indicates the total number of observations per group

Table 12: Gestation and parturition data (±SD) for all dose groups; n=(x).

Endpoint (Historical*)	Exposed Parents				F1 Offspring			
	Controls	Low-Dose	Mid-Dose	High-Dose	Controls	Low-Dose	Mid-Dose	High-Dose
Mating Index	90.3 (31)	93.5 (31)	90.3 (31)	96.7 (30)	100.0 (28)	100.0 (28)	96.4 (28)	92.9 (28)
Gestation Index	78.6 (28)	89.7 (29)	85.7 (28)	89.7 (29)	78.6 (28)	89.3 (28)	**100.0Ŧ** (27)	**100.0Ŧ** (26)
Live-born Index	97.5 ± 5.0 (22)	98.6 ± 3.5 (26)	94.8 ± 21.8 (24)	95.8 ± 13.4 (26)	99.5 ± 1.7 (22)	97.3 ± 7.1 (25)	99.5 ± 1.6 (27)	98.8 ± 2.9 (26)
Gestation Period (22.3 days)	22.6 ± 0.6 (18)	22.2 ± 0.5 (25)	22.6 ± 0.6 (21)	22.6 ± 0.6 (20)	22.0 ± 0.6 (20)	22.0 ± 0.7 (22)	21.9 ± 0.6 (24)	21.8 ± 0.4 (18)
Litter Size (14.7 Pups)	13.4 ± 4.4 (22)	12.5 ± 2.4 (26)	10.7 ± 3.7 (24)	11.2 ± 3.0 (26)	15.0 ± 3.6 (22)	13.6 ± 5.2 (22)	14.7 ± 1.9 (27)	15.3 ± 1.7 (26)
Sex Ratio (50% males)	51.0 ± 9.8 (22)	50.1 ± 15.3 (26)	49.8 ± 20.5 (24)	54.1 ± 16.5 (26)	49.3 ± 18.5 (14)	53.4 ± 15.7 (15)	45.6 ± 15.6 (17)	49.8 ± 13.2 (17)

***** Sharp, P., La Regina, M. The Laboratory Rat, CRC Press LLC, 1998

Ŧ Pearson Chi-square test indicated statistically significant differences (increases in the proportion of pregnant dams producing live born offspring) between the mid and high-dose groups and controls, which were validated by pair-wise comparisons using Fisher's exact test.

Table 13: Developmental Data (±SD) for offspring; n=(x).

Endpoint (Historical*)	F1 Offspring				F2 Offspring			
	Controls	Low-Dose	Mid-Dose	High-Dose	Controls	Low-Dose	Mid-Dose	High-Dose
Viability Index (% living at PND 4)	99 ± 3 (22)	98 ± 5 (25)	99 ± 5 (21)	99 ± 4 (26)	99 ± 2 (22)	92 ± 28 (25)	96 ± 12 (26)	99 ± 5 (26)
Lactation Index (% living at PND 21)	99 ± 3 (22)	99 ± 2 (25)	96 ± 12 (21)	94 ± 18 (26)	100 ± 0 (22)	97 ± 7 (25)	95 ± 20 (25)	99 ± 4 (25)
Time to open eyes and ears (10 – 14 days)	13.7 ± 0.9 (22)	14.3 ± 1.1 (25)	14.1 ± 1.1 (21)	14.1 ± 0.8 (25)	14.3 ± 0.8 (22)	14.3 ± 1.2 (25)	14.4 ± 0.6 (26)	14.4 ± 3.1 (25)
Male Weight at PND 0 (6.70 ± 0.37 grams)	7.1 ± 0.8 (21)	7.0 ± 0.6 (25)	7.4 ± 0.7 (22)	7.4 ± 0.7 (26)	6.8 ± 0.6 (12)	7.0 ± 0.7 (15)	6.8 ± 0.6 (17)	6.5 ± 0.4 (17)
Female Weight at PND 0 (6.31 ± 0.36 grams)	6.7 ± 0.8 (21)	6.6 ± 0.7 (25)	7.0 ± 0.6 (22)	7.0 ± 0.7 (26)	6.7 ± 0.9 (13)	6.8 ± 0.8 (15)	6.4 ± 0.8 (17)	6.3 ± 0.4 (17)
Male Weight at PND 21 (56.8 ± 5.9 grams)	64.3 ± 5.9 (22)	63.4 ± 6.3 (26)	67.7 ± 4.5 (21)	65.1 ± 6.9 (25)	57.1 ± 6.0 (21)	55.1 ± 6.3 (22)	56.5 ± 6.6 (25)	56.4 ± 6.0 (25)
Female Weight at PND 21 (54.5 ± 5.6 grams)	62.2 ± 5.9 (22)	59.8 ± 7.1 (26)	64.1 ± 4.4 (21)	62.9 ± 6.2 (24)	55.4 ± 6.3 (22)	52.2 ± 6.8 (22)	54.2 ± 5.6 (25)	53.8 ± 4.9 (25)

***** Sharp, P., La Regina, M. The Laboratory Rat, CRC Press LLC, 1998

Table 14: Pregnancy Outcomes (±SD) per uterine implantation sites identified in (P1) dams (195-214 day old female rats) euthanized 5-8 weeks following a continuous 90-105 day exposure to GD 19, parturition and weaning; n=7.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Uterine implantation sites per dam	11.6 ± 4.8	12.6 ± 2.6	11.4 ± 2.6	13.0 ± 1.5
Pups per litter (living and stillborn)	11.0 ± 4.8	12.3 ± 2.6	10.1 ± 3.3	11.9 ± 1.6
Live-born pups per litter	10.9 ± 4.7	12.0 ± 2.3	10.0 ± 3.1	11.4 ± 1.4
Resorptions per litter	0.57 ± 0.79	0.29 ± 0.49	1.29 ± 1.38	1.14 ± 0.90
Percent of live births to uterine implantation sites	91.2 ± 11.9	96.0 ± 5.4	86.4 ± 13.0	88.0 ± 5.6

Table 15: Mean organ weights expressed as a percentage of body weight (±SD) for adult female rats (195-214 day old dams) euthanized 5-8 weeks following a continuous 90-105 day exposure to GD 19, parturition and weaning; n=8.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Brain	0.60 ± 0.04	0.64 ± 0.03	0.59 ± 0.04	0.61 ± 0.05
Heart	0.43 ± 0.04	0.45 ± 0.05	0.40 ± 0.03	0.39 ± 0.02
Left Kidney	0.36 ± 0.04	0.37 ± 0.04	0.37 ± 0.04	0.35 ± 0.01
Right Kidney	0.37 ± 0.03	0.37 ± 0.03	0.38 ± 0.03	0.35 ± 0.02
Spleen	0.17 ± 0.03	0.18 ± 0.02	0.18 ± 0.03	0.17 ± 0.02
Liver	3.32 ± 0.33	3.25 ± 0.35	3.29 ± 0.17	3.05 ± 0.29
Ovary	0.27 ± 0.10	0.28 ± 0.07	0.23 ± 0.03	0.25 ± 0.06

Table 16: Mean organ weights expressed as a percentage of body weight (±SD) for adult male rats (163-172 day old sires) euthanized 3-5 weeks following a continuous 90-day exposure; n=(x).

Endpoint	Group 1 Control (6)	Group 2 Low-Dose (7)	Group 3 Mid-Dose (10)	Group 4 High-Dose (11)
Brain	0.39 ± 0.03	0.38 ± 0.03	0.39 ± 0.03	0.39 ± 0.05
Heart	0.32 ± 0.03	0.29 ± 0.02	0.30 ± 0.02	0.35 ± 0.09
Left Kidney	0.34 ± 0.04	**0.29^{Ŧ} ± 0.02**	0.32 ± 0.03	**0.30^{Ŧ} ± 0.01**
Right Kidney	0.34 ± 0.03	**0.30^{Ŧ} ± 0.01**	0.31 ± 0.03	**0.30^{Ŧ} ± 0.02**
Spleen	0.16 ± 0.03	0.16 ± 0.02	0.14 ± 0.03	0.17 ± 0.06
Liver	2.56 ± 0.16	2.57 ± 0.23	2.57 ± 0.19	2.43 ± 0.23
Testes	0.90 ± 0.07	0.95 ± 0.09	0.87 ± 0.11	0.95 ± 0.10

Ŧ ANCOVA indicated statistically significant differences (decreases) between the mean kidney weights of exposed males from the low and high-dose groups compared to controls. The mid-dose group also showed a (non-significant) kidney weight reduction compared to controls.

Table 17: Mean organ weights expressed as a percentage of body weight (±SD) for juvenile F1 female offspring (25-36 day old pups), whose parents were exposed at least 71 days prior to their conception, and who themselves were exposed *in utero* to GD 19; n=8.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Brain	1.59 ± 0.05	1.53 ± 0.11	1.65 ± 0.05	1.63 ± 0.05
Heart	0.56 ± 0.08	0.59 ± 0.09	0.62 ± 0.06	0.69 ± 0.09
Left Kidney	0.61 ± 0.04	0.57 ± 0.04	0.55 ± 0.04	0.52 ± 0.02
Right Kidney	0.61 ± 0.05	0.58 ± 0.02	0.57 ± 0.04	0.53 ± 0.02
Spleen	0.40 ± 0.10	0.41 ± 0.05	0.47 ± 0.22	0.33 ± 0.04
Liver	4.22 ± 0.12	4.26 ± 0.21	4.05 ± 0.26	3.95 ± 0.25
Ovary	0.15 ± 0.05	0.18 ± 0.08	0.23 ± 0.06	0.27 ± 0.05

Table 18: Mean organ weights expressed as a percentage of body weight (±SD) for juvenile F1 male offspring (25-36 day old pups), whose parents were exposed at least 71 days prior to their conception, and who themselves were exposed *in utero* to GD 19; n=8 except where noted.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Brain	1.67 ± 0.06	1.64 ± 0.04	1.73 ± 0.08	1.75 ± 0.13
Heart	0.62 ± 0.07	0.61 ± 0.06	0.68 ± 0.06	0.76 ± 0.10
Left Kidney	0.61 ± 0.05	0.58 ± 0.04	0.55 ± 0.04	0.51 ± 0.03
Right Kidney	0.63 ± 0.04	0.61 ± 0.04	0.58 ± 0.04	0.53 ± 0.03
Spleen	0.39 ± 0.06	0.43 ± 0.06	0.40 ± 0.07	0.37 ± 0.06
Liver	4.36 ± 0.23	4.18 ± 0.20	3.46 ± 1.41 (7)	3.79 ± 0.33
Ovary	0.78 ± 0.08	0.84 ± 0.09	0.91 ± 0.05	0.92 ± 0.06

Table 19: Mean organ weights expressed as a percentage of body weight (±SD) for adult F1 female offspring (94-105 day old females not selected for breeding), whose parents were exposed at least 71 days prior to their conception, and who themselves were exposed *in utero* to GD 19; n=8.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Brain	0.69 ± 0.06	0.68 ± 0.06	0.65 ± 0.04	0.67 ± 0.05
Heart	0.39 ± 0.04	0.38 ± 0.03	0.38 ± 0.05	0.41 ± 0.02
Left Kidney	0.36 ± 0.02	0.37 ± 0.04	0.34 ± 0.03	0.36 ± 0.01
Right Kidney	0.36 ± 0.03	0.38 ± 0.04	0.35 ± 0.03	0.37 ± 0.03
Spleen	0.23 ± 0.05	0.19 ± 0.05	0.18 ± 0.03	0.18 ± 0.03
Liver	2.89 ± 0.22	3.12 ± 0.24	2.87 ± 0.12	3.06 ± 0.15
Ovary	0.25 ± 0.08	0.24 ± 0.04	0.30 ± 0.08	0.26 ± 0.06

Table 20: Mean organ weights expressed as a percentage of body weight (±SD) for adult F1 male offspring (94-105 day old males not selected for breeding), whose parents were exposed at least 71 days prior to their conception, and who themselves were exposed *in utero* to GD 19; n=8, except where noted.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Brain	0.38 ± 0.04	0.38 ± 0.03	0.42 ± 0.02	0.40 ± 0.04
Heart	0.33 ± 0.04	0.34 ± 0.03	0.35 ± 0.03	0.33 ± 0.03
Left Kidney	0.34 ± 0.03	0.34 ± 0.02	0.36 ± 0.02	0.36 ± 0.04
Right Kidney	0.35 ± 0.04	0.35 ± 0.03	0.36 ± 0.02	0.36 ± 0.04
Spleen	0.17 ± 0.02	0.16 ± 0.02	0.16 ± 0.02	0.15 ± 0.02
Liver	3.25 ± 0.31	3.27 ± 0.29	3.07 ± 0.25	3.25 ± 0.43
Testes	0.90 ± 0.09	0.88 ± 0.09	0.98 ± 0.10	0.96 ± 0.15

Table 21: Mean organ weights expressed as a percentage of body weight (±SD) for adult F1 female offspring (114-126 day old dams), whose parents were exposed at least 71 days prior to their conception, and who themselves were exposed *in utero* to GD 19; n=8.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Brain	0.65 ± 0.05	0.65 ± 0.04	0.61 ± 0.05	0.64 ± 0.03
Heart	0.44 ± 0.02	0.43 ± 0.03	0.44 ± 0.03	0.45 ± 0.03
Left Kidney	0.42 ± 0.04	0.42 ± 0.04	0.41 ± 0.04	0.40 ± 0.02
Right Kidney	0.42 ± 0.03	0.43 ± 0.04	0.42 ± 0.06	0.41 ± 0.03
Spleen	0.20 ± 0.03	0.21 ± 0.03	0.18 ± 0.03	0.21 ± 0.03
Liver	3.68 ± 0.21	**4.56Ŧ ± 0.47**	3.79 ± 0.41	**4.39Ŧ ± 0.36**
Ovary	0.30 ± 0.08	0.31 ± 0.12	0.24 ± 0.08	0.34 ± 0.05

Ŧ ANCOVA indicated statistically significant differences (increases) between mean liver weights of 1st generation female breeders from the low and high-dose groups compared to controls. The mid-dose group also showed a (non-significant) liver increase compared to controls.

Table 22: Mean organ weights expressed as a percentage of body weight (±SD) for adult F1 male offspring (114-124 day old sires), whose parents were exposed at least 71 days prior to their conception, and who themselves were exposed *in utero* to GD 19; n=8.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Brain	0.37 ± 0.03	0.35 ± 0.05	0.35 ± 0.03	0.32 ± 0.13
Heart	0.29 ± 0.02	0.29 ± 0.02	0.32 ± 0.03	0.32 ± 0.03
Left Kidney	0.33 ± 0.03	0.34 ± 0.03	0.34 ± 0.02	0.34 ± 0.03
Right Kidney	0.34 ± 0.05	0.35 ± 0.04	0.35 ± 0.02	0.34 ± 0.03
Spleen	0.14 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.02
Liver	2.92 ± 0.27	3.18 ± 0.32	3.02 ± 0.25	3.01 ± 0.27
Testes	0.87 ± 0.09	0.86 ± 0.11	0.83 ± 0.08	0.90 ± 0.10

Table 23: Mean organ weights expressed as a percentage of body weight (±SD) for unexposed, juvenile F2 female offspring (23-28 day old pups), whose parents were exposed *in utero* to GD 19; n=8.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Brain	1.53 ± 0.07	1.48 ± 0.06	1.48 ± 0.08	1.48 ± 0.07
Heart	0.45 ± 0.06	0.38 ± 0.06	0.44 ± 0.04	0.40 ± 0.07
Left Kidney	0.64 ± 0.04	0.63 ± 0.05	0.63 ± 0.04	0.64 ± 0.04
Right Kidney	0.66 ± 0.03	0.64 ± 0.04	0.64 ± 0.05	0.66 ± 0.04
Spleen	0.46 ± 0.06	0.41 ± 0.05	**0.40Ŧ ± 0.09**	0.42 ± 0.06
Liver	4.51 ± 0.26	4.39 ± 0.28	4.24 ± 0.26	4.23 ± 0.19
Ovaries	0.19 ± 0.05	0.18 ± 0.04	0.24 ± 0.03	0.19 ± 0.03

Ŧ ANCOVA indicated a statistically significant difference (decrease) between the mean spleen weight of 2nd generation female pups from the mid-dose group compared to controls. The low and high-dose groups also showed (non-significant) spleen weight reductions compared to controls.

Table 24: Mean organ weights expressed as a percentage of body weight (±SD) for unexposed, juvenile F2 male offspring (23-28 day old pups), whose parents were exposed *in utero* to GD 19; n=8.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Brain	1.60 ± 0.06	1.54 ± 0.06	1.55 ± 0.07	1.58 ± 0.07
Heart	0.48 ± 0.07	0.39 ± 0.06	0.48 ± 0.06	0.42 ± 0.08
Left Kidney	0.62 ± 0.05	0.65 ± 0.08	0.60 ± 0.04	0.64 ± 0.03
Right Kidney	0.64 ± 0.06	0.68 ± 0.09	0.65 ± 0.05	0.67 ± 0.03
Spleen	0.47 ± 0.05	0.40 ± 0.05	0.39 ± 0.06	0.42 ± 0.06
Liver	4.44 ± 0.23	4.31 ± 0.44	4.16 ± 0.24	4.17 ± 0.22
Testes	0.76 ± 0.10	0.58 ± 0.08	0.73 ± 0.09	0.67 ± 0.05

Table 25: Mean organ weights expressed as a percentage of body weight (±SD) for unexposed, adult F2 female offspring (93-101 day old rats), whose parents were exposed *in utero* to GD 19; n=8.

Endpoint	**Group 1** Control	**Group 2** Low-Dose	**Group 3** Mid-Dose	**Group 4** High-Dose
Brain	0.69 ± 0.04	0.69 ± 0.07	0.65 ± 0.06	0.73 ± 0.05
Heart	0.39 ± 0.04	0.39 ± 0.03	0.39 ± 0.03	0.40 ± 0.04
Left Kidney	0.36 ± 0.01	0.35 ± 0.02	0.35 ± 0.03	0.37 ± 0.02
Right Kidney	0.37 ± 0.03	0.36 ± 0.01	0.36 ± 0.03	0.38 ± 0.02
Spleen	0.20 ± 0.01	0.21 ± 0.02	0.19 ± 0.02	0.20 ± 0.02
Liver	2.86 ± 0.16	3.02 ± 0.26	2.90 ± 0.23	2.98 ± 0.16
Ovaries	0.28 ± 0.07	0.28 ± 0.13	0.20 ± 0.04	0.25 ± 0.06

Table 26: Mean organ weights expressed as a percentage of body weight (±SD) for unexposed, adult F2 male offspring (86-99 day old rats), whose parents were exposed *in utero* to GD 19; n=8.

Endpoint	**Group 1** Control	**Group 2** Low-Dose	**Group 3** Mid-Dose	**Group 4** High-Dose
Brain	0.42 ± 0.04	0.43 ± 0.02	0.42 ± 0.04	0.46 ± 0.04
Heart	0.34 ± 0.02	0.35 ± 0.03	0.36 ± 0.04	0.38 ± 0.05
Left Kidney	0.36 ± 0.04	0.38 ± 0.03	0.38 ± 0.03	0.39 ± 0.03
Right Kidney	0.37 ± 0.05	0.38 ± 0.03	0.37 ± 0.04	0.39 ± 0.04
Spleen	0.17 ± 0.02	0.16 ± 0.02	0.16 ± 0.02	0.17 ± 0.02
Liver	3.11 ± 0.21	3.06 ± 0.27	3.18 ± 0.15	3.08 ± 0.28
Testes	0.93 ± 0.06	1.00 ± 0.06	0.95 ± 0.15	1.03 ± 0.08

Table 27: Hematology values (±SD) measured in adult P1 female rats (195-214 day old dams) euthanized 5-8 weeks following a continuous 90-105 day exposure to GD 19, parturition and weaning; n=8. The standard reference ranges for CD® IGS adult female rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
WBC (3.9 – 17.2 x 10^3/µL)	6.3 ± 2.4	6.2 ± 1.7	7.7 ± 1.3	6.1 ± 2.9
Lymphocytes (1.2 – 10.7 x 10^3/µL)	3.4 ± 1.5	2.8 ± 1.1	3.5 ± 0.9	2.6 ± 1.2
% Lymphocytes	53 ± 6	44 ± 7	46 ± 6	44 ± 5
Monocytes (0.10 – 0.90 x 10^3/µL)	0.55 ± 0.28	0.53 ± 0.26	0.65 ± 0.41	0.58 ± 0.44
% Monocytes	9 ± 3	9 ± 4	8 ± 4	9 ± 3
Neutrophils (0.5 – 8.1 x 10^3/µL)	2.4 ± 0.8	2.7 ± 0.7	3.3 ± 0.6	2.8 ± 1.4
% Neutrophils	38 ± 4	44 ± 4	44 ± 7	**46₸ ± 5**
Eosinophils (0.10 – 0.40 x 10^3/µL)	0.06 ± 0.04	0.12 ± 0.09	0.13 ± 0.07	0.05 ± 0.03
% Eosinophils	1.1 ± 0.7	1.9 ± 1.2	1.8 ± 1.2	1.3 ± 1.3
Basophils (≤ 0.20 x 10^3/µL)	0.00 ± 0.00	0.01 ± 0.01	0.03 ± 0.03	0.01 ± 0.01
% Basophils	0.1 ± 0.1	0.2 ± 0.1	0.4 ± 0.5	0.4 ± 0.4
RBC (5.1 – 9.0 x 10^6/µL)	7.7 ± 0.4	8.1 ± 0.3	**8.4₸ ± 0.3**	7.8 ± 0.6
% RDW	17.1 ± 1.0	17.3 ± 1.1	18.3 ± 2.0	17.2 ± 0.4
HB (8.6 – 16.3 x 10^6/µL)	15.6 ± 1.1	16.4 ± 0.7	**17.3₸ ± 0.8**	15.2 ± 1.2
% Hematocrit (28 – 55)	51 ± 2	54 ± 2	56 ± 4	51 ± 4
MCV (18 – 21 pg)	67 ± 2	67 ± 2	67 ± 2	66 ± 2
MCH (18 – 21 pg)	20 ± 1	20 ± 0.4	21 + 1	20 ± 1
MCHC (29 – 37 g/dL)	30 ± 2	30 ± 0.3	31 ± 1	30 ± 1
PLT (380 – 1210 x 10^3/µL)	1237 ± 185	765 ± 349	1011 ± 247	757 ± 446

₸ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Table 28: Hematology values (±SD) measured in adult P1 male rats (163-172 day old sires) euthanized 3-5 weeks following a continuous 90-day exposure; n=(x). The standard reference ranges for CD® IGS adult male rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (7)	Group 2 Low-Dose (8)	Group 3 Mid-Dose (9)	Group 4 High-Dose (9)
WBC (3.9 – 17.2 x 10^3/µL)	10.0 ± 4.6	7.2 ± 2.2	5.8 ± 2.1	6.8 ± 1.8
Lymphocytes (1.2 – 10.7 x 10^3/µL)	5.9 ± 4.5	2.7 ± 1.1	2.3 ± 0.7	3.0 ± 1.0
% Lymphocytes	54 ± 18	37 ± 8	40 ± 7	43 ± 6
Monocytes (0.10 – 0.90 x 10^3/µL)	0.47 ± 0.26	0.77 ± 0.35	0.57 ± 0.27	0.57 ± 0.22
% Monocytes	5 ± 1	**10^{Ψ} ± 2**	**10^{Ψ} ± 4**	**8^{Ψ} ± 2**
Neutrophils (0.5 – 8.1 x 10^3/µL)	3.6 ± 1.4	3.7 ± 1.1	2.9 ± 1.2	3.2 ± 0.8
% Neutrophils	41 ± 19	52 ± 10	49 ± 6	47 ± 6
Eosinophils (0.10 – 0.40 x 10^3/µL)	0.06 ± 0.05	0.04 ± 0.04	0.04 ± 0.03	0.08 ± 0.06
% Eosinophils	0.5 ± 0.3	0.6 ± 0.5	0.7 ± 0.5	1.2 ± 1.0
Basophils (≤ 0.20 x 10^3/µL)	0.01 ± 0.02	0.01 ± 0.01	0.00 ± 0.01	0.01 ± 0.02
% Basophils	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.3
RBC (5.1 – 9.0 x 10^6/µL)	8.5 ± 0.5	8.9 ± 0.3 (7)	8.9 ± 0.5	9.0 ± 0.6
% RDW	16.6 ± 0.8	16.2 ± 0.9	16.3 ± 0.5	17.0 ± 0.8
HB (8.6 – 16.3 x 10^6/µL)	15.0 ± 1.3	**$17.0^{Ŧ}$ ± 0.6**	16.2 ± 0.7	**$17.8^{Ŧ}$ ± 1.4**
% Hematocrit (28 – 55)	50 ± 4	55 ± 3	54 ± 3	55 ± 4
MCV (18 – 21 pg)	59 ± 2	62 ± 3	61 ± 1	60 ± 2
MCH (18 – 21 pg)	18 ± 1	19 ± 1	18 ± 1	**20^{Ψ} ± 1**
MCHC (29 – 37 g/dL)	30 ± 1	31 ± 1	30 ± 1	**$33^{Ŧ}$ ± 1**
PLT (380 – 1210 x 10^3/µL)	937 ± 158	1056 ± 81	1064 ± 85	1087 ± 70

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 29: Hematology values (±SD) measured in juvenile F1 female offspring (26-36 day old rat pups) whose parents were exposed at least 71 days prior to their conception, and who themselves were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS female rat pups are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (9)	Group 2 Low-Dose (8)	Group 3 Mid-Dose (9)	Group 4 High-Dose (8)
WBC (3.9 – 17.2 x 10^3/µL)	5.4 ± 1.4	6.2 ± 1.8	**7.8$^{\Psi}$ ± 2.5**	**8.5$^{\Psi}$ ± 2.4**
Lymphocytes (1.2 – 10.7 x 10^3/µL)	3.0 ± 1.0	3.9 ± 1.1	4.5 ± 1.7	**5.0$^{\Psi}$ ± 1.7**
% Lymphocytes	56 ± 8	63 ± 7	57 ± 9	58 ± 6
Monocytes (0.10 – 0.90 x 10^3/µL)	0.39 ± 0.16	0.48 ± 0.32	0.51 ± 0.18	0.55 ± 0.27
% Monocytes	7 ± 2	7 ± 4	7 ± 1	6 ± 2
Neutrophils (0.5 – 8.1 x 10^3/µL)	1.9 ± 0.5	1.8 ± 0.6	2.7 ± 1.2	2.9 ± 0.8
% Neutrophils	36 ± 8	29 ± 5	34 ± 8	35 ± 6
Eosinophils (0.10 – 0.40 x 10^3/µL)	0.05 ± 0.04	0.02 ± 0.02	0.08 ± 0.08	0.06 ± 0.06
% Eosinophils	0.9 ± 0.8	0.4 ± 0.3	1.5 ± 1.8	1.0 ± 1.1
Basophils (≤ 0.20 x 10^3/µL)	0.02 ± 0.02	0.01 ± 0.01	0.03 ± 0.04	0.02 ± 0.03
% Basophils	0.3 ± 0.3	0.1 ± 0.1	0.6 ± 0.8	0.3 ± 0.5
RBC (5.1 – 9.0 x 10^6/µL)	5.2 ± 0.4	5.2 ± 0.7	5.7 ± 0.4	**5.9^{Ŧ} ± 0.2**
% RDW	28.9 ± 3.5	28.7 ± 3.5	26.6 ± 3.1	**20.7^{Ŧ} ± 2.2**
HB (8.6 – 16.3 x 10^6/µL)	10.4 ± 1.2	10.2 ± 1.5	11.7 ± 0.9	12.0 ± 0.7
% Hematocrit (28 – 55)	33 ± 4	35 ± 5	**38$^{\Psi}$ ± 2**	**41$^{\Psi}$ ± 2**
MCV (18 – 21 pg)	63 ± 6	66 ± 6	67 ± 3	69 ± 5
MCH (18 – 21 pg)	20 ± 2	20 ± 2	21 + 1	20 ± 1
MCHC (29 – 37 g/dL)	32 ± 1	30 ± 2	31 ± 1	30 ± 1
PLT (380 – 1210 x 10^3/µL)	894 ± 378	704 ± 504	1219 ± 106	825 ± 366

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 30: Hematology values (±SD) measured in juvenile F1 male offspring (26-36 day old rat pups) whose parents were exposed at least 71 days prior to their conception, and who themselves were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS male rat pups are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (8)	Group 2 Low-Dose (8)	Group 3 Mid-Dose (9)	Group 4 High-Dose (10)
WBC (3.9 – 17.2 x 10^3/µL)	6.9 ± 1.6	6.5 ± 1.8	6.4 ± 2.2	9.0 ± 3.8
Lymphocytes (1.2 – 10.7 x 10^3/µL)	3.9 ± 0.8	3.9 ± 1.3	3.3 ± 1.0	5.0 ± 2.6
% Lymphocytes	57 ± 6	60 ± 4	52 ± 7	53 ± 8
Monocytes (0.10 – 0.90 x 10^3/µL)	0.45 ± 0.15	0.47 ± 0.23	0.57 ± 0.34	0.74 ± 0.44
% Monocytes	6 ± 2	7 ± 3	8 ± 3	8 ± 3
Neutrophils (0.5 – 8.1 x 10^3/µL)	2.5 ± 0.8	2.0 ± 0.5	2.5 ± 1.1	3.2 ± 1.0
% Neutrophils	36 ± 6	32 ± 3	38 ± 7	37 ± 7
Eosinophils (0.10 – 0.40 x 10^3/µL)	0.08 ± 0.06	0.04 ± 0.05	0.07 ± 0.03	0.06 ± 0.10
% Eosinophils	1.1 ± 0.8	0.6 ± 0.7	1.3 ± 1.0	1.4 ± 2.6
Basophils (≤ 0.20 x 10^3/µL)	0.02 ± 0.04	0.02 ± 0.03	0.02 ± 0.02	0.01 ± 0.02
% Basophils	0.3 ± 0.4	0.3 ± 0.5	0.3 ± 0.3	0.3 ± 0.6
RBC (5.1 – 9.0 x 10^6/µL)	5.1 ± 0.4	5.1 ± 0.4	5.3 ± 0.6	**5.7$^{\Psi}$ ± 0.1**
% RDW	30.1 ± 3.0	28.4 ± 4.4	26.7 ± 3.1	**22.3^{Ŧ} ± 2.4**
HB (8.6 – 16.3 x 10^6/µL)	10.0 ± 1.0	10.2 ± 1.4	10.9 ± 1.5	**12.1$^{\Psi}$ ± 0.5**
% Hematocrit (28 – 55)	32 ± 3	34 ± 5	36 ± 6	**41$^{\Psi}$ ± 3**
MCV (18 – 21 pg)	63 ± 4	65 ± 7	67 ± 4	**72^{Ŧ} ± 4**
MCH (18 – 21 pg)	20 ± 1	20 ± 2	20 ± 1	21 ± 1
MCHC (29 – 37 g/dL)	31 ± 1	30 ± 1	31 ± 1	30 ± 1
PLT (380 – 1210 x 10^3/µL)	1061 ± 199	1081 ± 168	1223 ± 305	1139 ± 154

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 31: Hematology values (±SD) measured in adult F1 female offspring (94-105 day old females not selected for breeding) whose parents were exposed at least 71 days prior to their conception, and who were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS adult female rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (7)	Group 2 Low-Dose (7)	Group 3 Mid-Dose (6)	Group 4 High-Dose (6)
WBC (3.9 – 17.2 x 10^3/µL)	8.7 ± 2.5	8.3 ± 3.0	7.7 ± 2.7	10.2 ± 2.8
Lymphocytes (1.2 – 10.7 x 10^3/µL)	5.0 ± 1.8	4.8 ± 2.1	4.6 ± 1.7	5.9 ± 1.4
% Lymphocytes	57 ± 7	54 ± 3 (6)	59 ± 5	58 ± 5
Monocytes (0.10 – 0.90 x 10^3/µL)	0.65 ± 0.32	0.58 ± 0.22	0.53 ± 0.18	0.66 ± 0.07 (5)
% Monocytes	7 ± 2	7 ± 2	7 ± 1	8 ± 2
Neutrophils (0.5 – 8.1 x 10^3/µL)	2.9 ± 0.9	2.8 ± 1.1	2.5 ± 0.9	3.3 ± 1.0
% Neutrophils	34 ± 9	37 ± 3 (6)	32 ± 5	32 ± 4
Eosinophils (0.10 – 0.40 x 10^3/µL)	0.11 ± 0.08	0.10 ± 0.08	0.08 ± 0.07	0.09 ± 0.07 (5)
% Eosinophils	1.3 ± 1.1	1.2 ± 0.9	1.0 ± 0.7	0.9 ± 0.7 (5)
Basophils (≤ 0.20 x 10^3/µL)	0.04 ± 0.03	0.03 ± 0.03	0.02 ± 0.03	0.02 ± 0.03 (5)
% Basophils	0.5 ± 0.5	0.3 ± 0.3	0.3 ± 0.4	0.2 ± 0.3 (5)
RBC (5.1 – 9.0 x 10^6/µL)	8.0 ± 0.7	7.9 ± 0.5	8.0 ± 0.4	8.1 ± 0.1
% RDW	14.7 ± 1.0	14.7 ± 0.4	14.2 ± 0.5	14.6 ± 0.5
HB (8.6 – 16.3 x 10^6/µL)	15.7 ± 0.8	15.5 ± 1.1	15.5 ± 0.7	15.9 ± 0.6
% Hematocrit (28 – 55)	53 ± 4	52 ± 3	53 ± 3	54 ± 1
MCV (18 – 21 pg)	66 ± 2	67 ± 3	66 ± 2	66 ± 1
MCH (18 – 21 pg)	20 ± 1 (6)	20 ± 1	20 + 1	20 ± 1
MCHC (29 – 37 g/dL)	30 ± 0 (6)	30 ± 1	29 ± 1	30 ± 1
PLT (380 – 1210 x 10^3/µL)	1144 ± 101 (6)	1010 ± 193	1161 ± 169	1190 ± 138

Table 32: Hematology values (±SD) measured adult F1 male offspring (94-105 day old males not selected for breeding) whose parents were exposed at least 71 days prior to their conception, and who were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS adult male rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (8)	Group 2 Low-Dose (8)	Group 3 Mid-Dose (7)	Group 4 High-Dose (7)
WBC (3.9 – 17.2 x 10^3/µL)	10.8 ± 3.3	10.8 ± 3.6	9.7 ± 1.3	10.3 ± 2.1
Lymphocytes (1.2 – 10.7 x 10^3/µL)	5.8 ± 1.9	5.7 ± 2.0	5.0 ± 0.8	5.7 ± 1.3
% Lymphocytes	54 ± 6	53 ± 6	52 ± 5	55 ± 5
Monocytes (0.10 – 0.90 x 10^3/µL)	0.86 ± 0.27	0.85 ± 0.26	0.66 ± 0.29	0.84 ± 0.35
% Monocytes	8 ± 2	8 ± 1	7 ± 3	8 ± 3
Neutrophils (0.5 – 8.1 x 10^3/µL)	4.0 ± 1.5	4.1 ± 1.5	3.9 ± 0.8	3.7 ± 0.7
% Neutrophils	37 ± 7	38 ± 4	40 ± 5	36 ± 3
Eosinophils (0.10 – 0.40 x 10^3/µL)	0.09 ± 0.07	0.05 ± 0.05 (7)	0.07 ± 0.05 (6)	0.04 ± 0.01 (6)
% Eosinophils	0.8 ± 0.6	0.4 ± 0.3 (7)	1.4 ± 1.9	0.5 ± 0.4
Basophils (≤ 0.20 x 10^3/µL)	0.01 ± 0.02	0.00 ± 0.00 (7)	0.03 ± 0.07	0.01 ± 0.02
% Basophils	0.1 ± 0.1	0.0 ± 0.3 (7)	0.3 ± 0.7	0.1 ± 0.2
RBC (5.1 – 9.0 x 10^6/µL)	8.3 ± 0.2	8.3 ± 0.6	8.5 ± 0.2	8.4 ± 0.4
% RDW	15.2 ± 0.5	15.2 ± 0.5	15.5 ± 0.5	15.5 ± 0.8
HB (8.6 – 16.3 x 10^6/µL)	15.9 ± 0.6	15.8 ± 0.9	16.4 ± 0.5	16.0 ± 0.7
% Hematocrit (28 – 55)	54 ± 1	53 ± 3	56 ± 1	54 ± 2
MCV (18 – 21 pg)	65 ± 1	65 ± 2	65 ± 2	65 ± 2
MCH (18 – 21 pg)	19 ± 1	19 ± 1	19 + 1	19 ± 0 (6)
MCHC (29 – 37 g/dL)	30 ± 1	30 ± 1	30 ± 1	29 ± 1
PLT (380 – 1210 x 10^3/µL)	1302 ± 92 (7)	1342 ± 92 (7)	1129 ± 47 (6)	1246 ± 95

Table 33: Hematology values (±SD) measured in adult F1 female offspring (114-125 day old dams), whose parents were exposed at least 71 days prior to their conception, and who were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS adult female rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (8)	Group 2 Low-Dose (8)	Group 3 Mid-Dose (8)	Group 4 High-Dose (7)
WBC (3.9 – 17.2 x 10^3/µL)	7.2 ± 2.0	8.9 ± 3.9	7.4 ± 2	8.8 ± 1.5
Lymphocytes (1.2 – 10.7 x 10^3/µL)	3.1 ± 1.0	3.4 ± 2.3	2.7 ± 0.8	3.3 ± 0.7
% Lymphocytes	42 ± 6	37 ± 11	37 ± 7	38 ± 6
Monocytes (0.10 – 0.90 x 10^3/µL)	0.77 ± 0.28	0.68 ± 0.34	0.94 ± 0.34	0.74 ± 0.11
% Monocytes	11 ± 3	8 ± 2	13 ± 3	9 ± 1
Neutrophils (0.5 – 8.1 x 10^3/µL)	3.2 ± 0.9	4.5 ± 1.8	3.6 ± 1.1	4.6 ± 1.2
% Neutrophils	45 ± 5	53 ± 12	49 ± 8	52 ± 7
Eosinophils (0.10 – 0.40 x 10^3/µL)	0.13 ± 0.10	0.18 ± 0.17	0.10 ± 0.11	0.08 ± 0.07
% Eosinophils	1.8 ± 1.2	1.8 ± 1.6	1.4 ± 1.6	0.9 ± 0.8
Basophils (≤ 0.20 x 10^3/µL)	0.02 ± 0.04	0.05 ± 0.06	0.01 ± 0.01	0.01 ± 0.01
% Basophils	0.3 ± 0.5	0.5 ± 0.6	0.1 ± 0.2	0.1 ± 0.0
RBC (5.1 – 9.0 x 10^6/µL)	8.3 ± 0.5	7.8 ± 0.5	8.5 ± 0.7	8.3 ± 0.3
% RDW	16.4 ± 0.8	16.3 ± 0.9	16.5 ± 0.7	16.1 ± 0.9
HB (8.6 – 16.3 x 10^6/µL)	17.2 ± 0.5	16.6 ± 0.7	18.1 ± 1.3	17.7 ± 0.7
% Hematocrit (28 – 55)	56 ± 3	53 ± 3	59 ± 5	57 ± 1
MCV (18 – 21 pg)	67 ± 2	68 ± 1	70 ± 3	69 ± 1
MCH (18 – 21 pg)	21 ± 1	21 ± 1	21 + 1	21 ± 1
MCHC (29 – 37 g/dL)	31 ± 1	31 ± 1	31 ± 1	31 ± 1
PLT (380 – 1210 x 10^3/µL)	1225 ± 154 (7)	1219 ± 191	1295 ± 133	1328 ± 183

Table 34: Hematology values (±SD) measured in adult F1 male offspring (114-125 day old sires), whose parents were exposed at least 71 days prior to their conception, and who were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS adult male rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (8)	Group 2 Low-Dose (8)	Group 3* Mid-Dose (7)	Group 4 High-Dose (6)
WBC (3.9 – 17.2 x 10^3/µL)	8.9 ± 1.4	11.1 ± 2.6	9.9 ± 2.1	9.4 ± 3.1
Lymphocytes (1.2 – 10.7 x 10^3/µL)	4.7 ± 0.9	5.2 ± 1.8	**3.9Ŧ ± 1.2**	4.2 ± 1.8
% Lymphocytes	52 ± 5	46 ± 9	**40Ŧ ± 10**	42 ± 10
Monocytes (0.10 – 0.90 x 10^3/µL)	0.65 ± 0.27	0.79 ± 0.25	0.94 ± 0.32	0.78 ± 0.23
% Monocytes	7.3 ± 2.7	7.1 ± 1.9	**9.5Ŧ ± 3.0**	8.8 ± 2.7
Neutrophils (0.5 – 8.1 x 10^3/µL)	3.5 ± 0.6	5.0 ± 1.2	5.0 ± 1.8	4.5 ± 1.2
% Neutrophils	40 ± 4	45 ± 8	50 ± 12	48 ± 8
Eosinophils (0.10 – 0.40 x 10^3/µL)	0.06 ± 0.05	0.16 ± 0.15	0.06 ± 0.05	0.04 ± 0.05
% Eosinophils	0.7 ± 0.7	1.6 ± 1.6	0.6 ± 0.4	0.4 ± 0.4
Basophils (≤ 0.20 x 10^3/µL)	0.01 ± 0.01	0.04 ± 0.04	0.00 ± 0.00	0.01 ± 0.01
% Basophils	0.1 ± 0.2	0.4 ± 0.4	0.02 ± 0.03	0.1 ± 0.1
RBC (5.1 – 9.0 x 10^6/µL)	8.1 ± 0.6	8.3 ± 0.5	8.5 ± 0.3	8.4 ± 0.4
% RDW	15.6 ± 0.6	16.0 ± 0.8	15.7 ± 0.8	16.6 ± 0.8
HB (8.6 – 16.3 x 10^6/µL)	15.5 ± 1.5	15.9 ± 1.1	**16.5Ŧ ± 0.7**	16.3 ± 1.2
% Hematocrit (28 – 55)	50 ± 3	51 ± 3	52 ± 3	49 ± 2
MCV (18 – 21 pg)	62 ± 1	61 ± 3	61 ± 3	58 ± 2
MCH (18 – 21 pg)	19 ± 1	19 ± 1	19 ± 1	19 ± 3
MCHC (29 – 37 g/dL)	31 ± 1	32 ± 1	32 ± 2	33 ± 4
PLT (380 – 1210 x 10^3/µL)	1082 ± 133	1107 ± 87 (7)	1176 ± 108	1206 ± 134

*****Blood samples from the mid-dose group were noted with high incidents of hemolysis and clotting.

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Table 35: Hematology values (±SD) measured in juvenile F2 female offspring (22-27 day old rat pups), whose parents were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS female rat pups are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (11)	Group 2 Low-Dose (9)	Group 3 Mid-Dose (10)	Group 4 High-Dose (7)
WBC (3.9 – 17.2 x 10^3/µL)	6.0 ± 0.8	**4.3$^{\Psi}$ ± 2.4**	**4.3$^{\Psi}$ ± 1.1**	**3.9$^{\Psi}$ ± 1.5**
Lymphocytes (1.2 – 10.7 x 10^3/µL)	3.6 ± 0.4	**2.5$^{\Psi}$ ± 1.4**	**2.4$^{\Psi}$ ± 0.7**	**2.3$^{\Psi}$ ± 1.0**
% Lymphocytes	60 ± 4	57 ± 3	58 ± 9	57 ± 5
Monocytes (0.10 – 0.90 x 10^3/µL)	0.42 ± 0.18	**0.17$^{\text{Ŧ}}$ ± 0.10**	0.33 ± 0.17	**0.22$^{\text{Ŧ}}$ ± 0.14**
% Monocytes	7 ± 3	4 ± 2	8 ± 3	5 ± 2
Neutrophils (0.5 – 8.1 x 10^3/µL)	1.9 ± 0.4	1.6 ± 0.9	1.5 ± 0.5	1.4 ± 0.5
% Neutrophils	32 ± 4	38 ± 4	34 ± 6	37 ± 5
Eosinophils (0.10 – 0.40 x 10^3/µL)	0.05 ± 0.04	0.03 ± 0.03	0.04 ± 0.07	0.01 ± 0.01
% Eosinophils	0.8 ± 0.5	0.6 ± 0.6	0.9 ± 1.6	0.2 ± 0.4
Basophils (≤ 0.20 x 10^3/µL)	0.01 ± 0.02	0.00 ± 0.00	0.02 ± 0.03	0.00 ± 0.00
% Basophils	0.2 ± 0.2	0.1 ± 0.1	0.3 ± 0.5	0.1 ± 0.2
RBC (5.1 – 9.0 x 10^6/µL)	5.6 ± 0.5	**4.6$^{\text{Ŧ}}$ ± 0.6**	5.5 ± 0.4	5.3 ± 0.5
% RDW	29.4 ± 4.2	30.3 ± 5.3	33.9 ± 6.9	33.6 ± 5.6
HB (8.6 – 16.3 x 10^6/µL)	11.2 ± 0.9	**9.1$^{\text{Ŧ}}$ ± 1.0**	11.1 ± 1.2	10.4 ± 0.9
% Hematocrit (28 – 55)	38 ± 3	**31$^{\text{Ŧ}}$ ± 4**	38 ± 5	35 ± 3
MCV (18 – 21 pg)	69 ± 6	67 ± 8	69 ± 7	67 ± 4
MCH (18 – 21 pg)	20 ± 1	20 ± 2	20 + 2	20 ± 1
MCHC (29 – 37 g/dL)	29 ± 1	30 ± 2	30 ± 1	29 ± 1
PLT (380 – 1210 x 10^3/µL)	1031 ± 496	990 ± 147	1027 ± 151	1139 ± 121

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 36: Hematology values (±SD) measured in juvenile F2 male offspring (22-27 day old rat pups), whose parents were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS male rat pups are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (11)	Group 2 Low-Dose (8)	Group 3 Mid-Dose (8)	Group 4 High-Dose (8)
WBC (3.9 – 17.2 x 10^3/µL)	6.4 ± 1.4	**4.2Ŧ ± 1.3**	**4.1Ŧ ± 1.5**	4.7 ± 1.6
Lymphocytes (1.2 – 10.7 x 10^3/µL)	3.7 ± 1.1	**2.2Ŧ ± 0.7**	**2.3Ŧ ± 1.0**	2.6 ± 1.0
% Lymphocytes	57 ± 6	51 ± 6	55 ± 7	56 ± 6
Monocytes (0.10 – 0.90 x 10^3/µL)	0.38 ± 0.17	0.24 ± 0.09	0.22 ± 0.16	0.26 ± 0.13
% Monocytes	6 ± 2	6 ± 2	5 ± 2	5 ± 2
Neutrophils (0.5 – 8.1 x 10^3/µL)	2.3 ± 0.4	1.8 ± 0.5	1.6 ± 0.4	1.8 ± 0.6
% Neutrophils	36 ± 5	42 ± 5	40 ± 7	38 ± 7
Eosinophils (0.10 – 0.40 x 10^3/µL)	0.04 ± 0.05	0.06 ± 0.11	0.01 ± 0.02	0.01 ± 0.01
% Eosinophils	0.7 ± 0.9	1.0 ± 1.6	0.3 ± 0.4	0.2 ± 0.3
Basophils (≤ 0.20 x 10^3/µL)	0.01 ± 0.02	0.02 ± 0.03	0.01 ± 0.01	0.00 ± 0.00
% Basophils	0.2 ± 0.3	0.4 ± 0.5	0.2 ± 0.3	0.0 ± 0.0
RBC (5.1 – 9.0 x 10^6/µL)	5.2 ± 0.4	**4.5Ŧ ± 0.3**	5.4 ± 0.4	5.0 ± 0.4
% RDW	30.3 ± 5.2	28.8 ± 5.9	33.6 ± 5.8	36.5 ± 5.8
HB (8.6 – 16.3 x 10^6/µL)	10.5 ± 1.1	**9.2Ŧ ± 1.3**	10.7 ± 1.2	9.3 ± 1.0
% Hematocrit (28 – 55)	36 ± 3	31 ± 5	37 ± 4	32 ± 4
MCV (18 – 21 pg)	69 ± 3	69 ± 7	68 ± 5	64 ± 7
MCH (18 – 21 pg)	20 ± 1	21 ± 2	20 ± 2	19 ± 2
MCHC (29 – 37 g/dL)	29 ± 1	30 ± 1	29 ± 1	29 ± 1
PLT (380 – 1210 x 10^3/µL)	1206 ± 255 (10)	1063 ± 65	1182 ± 194	1052 ± 224

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Table 37: Hematology values (±SD) measured in adult F2 female offspring (86-98 day old rats), whose parents were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS adult female rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (8)	Group 2 Low-Dose (8)	Group 3 Mid-Dose (8)	Group 4 High-Dose (8)
WBC (3.9 – 17.2 x 10^3/µL)	8.8 ± 1.8	9.3 ± 2.3	8.4 ± 1.6	8.3 ± 2.1
Lymphocytes (1.2 – 10.7 x 10^3/µL)	5.3 ± 1.9	4.5 ± 0.8	4.2 ± 1.0	4.3 ± 1.1
% Lymphocytes	59 ± 11	49 ± 6	50 ± 5	52 ± 8
Monocytes (0.10 – 0.90 x 10^3/µL)	0.71 ± 0.24	0.91 ± 0.32	0.66 ± 0.18	0.60 ± 0.21 (7)
% Monocytes	8 ± 2	10 ± 3	8 ± 2	8 ± 2
Neutrophils (0.5 – 8.1 x 10^3/µL)	2.8 ± 0.7	3.8 ± 1.5	3.4 ± 0.8	3.3 ± 1.1
% Neutrophils	32 ± 10	40 ± 7	41 ± 6	39 ± 8
Eosinophils (0.10 – 0.40 x 10^3/µL)	0.02 ± 0.02	0.11 ± 0.10	0.08 ± 0.07	0.06 ± 0.09
% Eosinophils	0.3 ± 0.2	1.2 ± 1.1	1.0 ± 0.9	0.7 ± 1.0
Basophils (≤ 0.20 x 10^3/µL)	0.01 ± 0.01	0.00 ± 0.01	0.02 ± 0.03	0.01 ± 0.01
% Basophils	0.1 ± 0.1	0.0 ± 0.1	0.2 ± 0.4	0.1 ± 0.1
RBC (5.1 – 9.0 x 10^6/µL)	8.2 ± 0.4	8.0 ± 0.5	7.8 ± 0.4	8.0 ± 0.5
% RDW	15.8 ± 0.5	15.0 ± 0.7	14.9 ± 0.4	15.0 ± 0.4 (7)
HB (8.6 – 16.3 x 10^6/µL)	15.9 ± 0.9	15.9 ± 1.0	15.8 ± 0.9	15.7 ± 0.8
% Hematocrit (28 – 55)	52 ± 3	52 ± 2	51 ± 3	52 ± 3
MCV (18 – 21 pg)	63 ± 2	65 ± 3	65 ± 2	65 ± 2
MCH (18 – 21 pg)	20 ± 1	20 ± 1	20 + 0	20 ± 1
MCHC (29 – 37 g/dL)	31 ± 1	31 ± 0.7	31 ± 0	30 ± 1
PLT (380 – 1210 x 10^3/µL)	1143 ± 131	1074 ± 93	1094 ± 111	1153 ± 101

Table 38: Hematology values (±SD) measured in adult F2 male offspring (86-98 day old rats), whose parents were exposed *in utero* to GD 19; n=8, except where noted. The standard reference ranges for CD® IGS adult male rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
WBC ($3.9 – 17.2 \times 10^3/\mu L$)	11.6 ± 3.2	11.3 ± 1.9 (7)	11.9 ± 1.2	11.6 ± 2.9
Lymphocytes ($1.2 – 10.7 \times 10^3/\mu L$)	6.5 ± 2.3	5.3 ± 1.1 (7)	6.0 ± 1.0	6.2 ± 1.9
% Lymphocytes	54 ± 10	48 ± 10 (7)	50 ± 6	53 ± 6
Monocytes ($0.10 – 0.90 \times 10^3/\mu L$)	1.06 ± 0.27	1.12 ± 0.38 (7)	1.26 ± 0.31	0.89 ± 0.21
% Monocytes	10 ± 4	10 ± 3 (7)	11 ± 2	8 ± 2
Neutrophils ($0.5 – 8.1 \times 10^3/\mu L$)	4.0 ± 0.9	4.7 ± 1.6 (7)	4.5 ± 0.6	4.4 ± 1.4
% Neutrophils	35 ± 7	41 ±11 (7)	38 ± 5	38 ± 7
Eosinophils ($0.10 – 0.40 \times 10^3/\mu L$)	0.05 ± 0.03 (7)	0.13 ± 0.14 (7)	0.15 ± 0.16	0.08 ± 0.04
% Eosinophils	0.6 ± 0.4	1.1 ± 1.2 (7)	1.2 ± 1.2	0.7 ± 0.3
Basophils ($\leq 0.20 \times 10^3/\mu L$)	0.01 ± 0.02	0.02 ± 0.02 (7)	0.02 ± 0.03	0.01 ± 0.03
% Basophils	0.1 ± 0.1	0.2 ± 0.2 (7)	0.2 ± 0.3	0.1 ± 0.3
RBC ($5.1 – 9.0 \times 10^6/\mu L$)	8.3 ± 0.3	8.4 ± 0.2	8.2 ± 0.2 (7)	8.5 ± 0.3 (7)
% RDW	16.8 ± 0.7	17.2 ± 0.9	16.5 ± 0.4 (7)	17.6 ± 0.6
HB ($8.6 – 16.3 \times 10^6/\mu L$)	16.2 ± 0.7	16.7 ± 0.7	16.4 ± 0.7 (7)	16.7 ± 1.2
% Hematocrit (28 – 55)	52 ± 2	52 ± 1	53 ± 1 (7)	54 ± 3
MCV (18 – 21 pg)	62 ± 2	62 ± 2	64 ± 2 (7)	63 ± 2
MCH (18 – 21 pg)	20 ± 1	20 ± 1	20 + 1 (7)	19 ± 1
MCHC (29 – 37 g/dL)	31 ± 1	32 ± 1	31 ± 1 (7)	31 ± 1
PLT ($380 – 1210 \times 10^3/\mu L$)	1154 ± 136	1157 ± 71	1105 ± 143	1172 ± 138

Table 39: Serum chemistries (±SD) measured in adult P1 female rats (195-214 day old dams) euthanized 5-8 weeks following a continuous 90-105 day exposure to GD 19, parturition and weaning; n=(x). The standard reference ranges for CD® IGS adult female rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (7)	Group 2 Low-Dose (8)	Group 3 Mid-Dose (8)	Group 4 High-Dose (7)
TP (5.7 – 8.9 g/dL)	6.6 ± 0.5	6.8 ± 0.3	6.9 ± 0.3	7.0 ± 0.6
ALB (3.3 – 6.7 g/dL)	3.8 ± 0.3	3.9 ± 0.2 (7)	4 ± 0.2	3.8 ± 0.5
ALKP (90 – 205 U/L)	107 ± 29.2	122 ± 16 (7)	**83$^{\Psi}$ ± 11** (6)	**79$^{\Psi}$ ± 17** (6)
ALT (23 – 186 U/L)	48 ± 7	54 ± 8	54 ± 14 (7)	59 ± 17
AST (78 – 226 U/L)	80 ± 14	84 ± 23	85 ± 16 (7)	86 ± 19
BUN (10 – 25 mg/dL)	17 ± 2	19 ± 2	19 ± 4	20 ± 3
CHOL (47 – 92 mg/dL)	87 ± 24	71 ± 25	83 ± 20	76 ± 24
CK (117 – 531 U/L)	112 ± 29	123 ± 81 (7)	119 ± 44	140 ± 49
CREA (0.50 – 0.90 mg/dL)	0.46 ± 0.05	0.45 ± 0.05	0.48 ± 0.05	0.46 ± 0.05
GLU (81 – 185 mg/dL)	163 ± 41	144 ± 10	164 ± 36	148 ± 22
TBIL (0.10 – 1.00 mg/dL)	0.17 ± 0.08	0.25 ± 0.14	0.11 ± 0.04	0.21 ± 0.09
TRIG (30 – 205 mg/dL)	57 ± 21	56 ± 17	61 ± 20	65 ± 25
Na+ (140 – 156 mEq/L)	152 ± 2	150 ± 1	151 ± 2	151 ± 2
K+ (4.1 – 6.9 mEq/L)	6.2 ± 0.4	6.1 ± 0.3	5.9 ± 0.8	**7.1$^{\Psi}$ ± 1.0**
Cl- (95 – 111 mEq/L)	104 ± 2	105 ± 2	104 ± 2	105 ± 1

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 40: Serum chemistries (±SD) measured in adult P1 male rats (163-172 day old sires) euthanized 3-5 weeks following a continuous 90-day exposure; n=(x). The standard reference ranges for CD® IGS adult male rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (6)	Group 2 Low-Dose (6)	Group 3 Mid-Dose (4)	Group 4 High-Dose (5)
TP (5.7 – 8.9 g/dL)	6.3 ± 0.2	6.0 ± 0.2	6.3 ± 0.05	6.1 ± 0.5 (4)
ALB (3.3 – 6.7 g/dL)	3.5 ± 0.2	3.4 ± 0.2	3.4 ± 0.2	3.4 ± 0.1 (4)
ALKP (90 – 205 U/L)	106 ± 28	85 ± 12	87 ± 2 (3)	72 ± 8
ALT (23 – 186 U/L)	72 ± 14	50 ± 12	44 ± 10	54 ± 19
AST (78 – 226 U/L)	111 ± 29 (5)	84 ± 18	87 ± 14	83 ± 16
BUN (10 – 25 mg/dL)	19 ± 3	17 ± 2	19 ± 1	17 ± 1
CHOL (47 – 92 mg/dL)	46 ± 12	46 ± 14	53 ± 7	35 ± 7
CK (117 – 531 U/L)	120 ± 53	98 ± 14	152 ± 65	93 ± 22
CREA (0.50 – 0.90 mg/dL)	0.50 ± 0.09	0.55 ± 0.08	0.60 ± 0.08	0.52 ± 0.04
GLU (81 – 185 mg/dL)	156 ± 34	148 ± 36	162 ± 12	115 ± 10
TBIL (0.10 – 1.00 mg/dL)	0.12 ± 0.04	0.22 ± 0.08	0.13 ± 0.05	0.12 ± 0.04
TRIG (30 – 205 mg/dL)	57 ± 14	78 ± 14	65 ± 13	48 ± 11
Na+ (140 – 156 mEq/L)	154 ± 2	153 ± 2	155 ± 2	153 ± 2
K+ (4.1 – 6.9 mEq/L)	6.7 ± 0.4	6.4 ± 0.5	6.0 ± 0.3	7.13 ± 0.3 (4)
Cl- (95 – 111 mEq/L)	104 ± 2	106 ± 1	105 ± 1	106 ± 2

Table 41: Serum chemistries (±SD) measured in juvenile F1 female offspring (26-36 day old rat pups), whose parents were exposed at least 71 days prior to their conception, and who themselves were exposed *in utero* to GD 19; n=7. The standard reference ranges for CD® IGS female rat pups are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
TP (5.7 – 8.9 g/dL)	4.8 ± 0.3	5.1 ± 0.2	4.7 ± 0.2	4.9 ± 0.3
ALB (3.3 – 6.7 g/dL)	2.8 ± 0.1	3.1 ± 0.1	2.9 ± 0.1	2.7 ± 0.1
ALKP (90 – 205 U/L)	397 ± 48	385 ± 27	375 ± 26 (6)	420 ± 87
ALT (23 – 186 U/L)	45 ± 5	46 ± 5	48 ± 7	**57Ŧ ± 6**
AST (78 – 226 U/L)	96 ± 11	86 ± 17	82 ± 5	90 ± 8
BUN (10 – 25 mg/dL)	13 ± 4	11 ± 2	9 ± 2.1	9 ± 2
CHOL (47 – 92 mg/dL)	74 ± 7	75 ± 11	74 ± 8	78 ± 22
CK (117 – 531 U/L)	224 ± 73	192 ± 72	175 ± 39	200 ± 41
CREA (0.50 – 0.90 mg/dL)	0.20 ± 0.00	0.23 ± 0.08	0.20 ± 0.04	0.23 ± 0.08
GLU (81 – 185 mg/dL)	184 ± 19	180 ± 6	174 ± 9	**158Ψ ± 9**
TBIL (0.10 – 1.00 mg/dL)	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
TRIG (30 – 205 mg/dL)	49 ± 24	44 ± 12	36 ± 7	35 ± 9
Na+ (140 – 156 mEq/L)	147 ± 2	148 ± 1	146 ± 1	146 ± 2
K+ (4.1 – 6.9 mEq/L)	7.0 ± 0.3	6.8 ± 0.3	6.9 ± 0.6	6.9 ± 0.4
Cl- (95 – 111 mEq/L)	107 ± 2	107 ± 1	106 ± 1	**103Ŧ ± 2**

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 42: Serum chemistries (±SD) measured in juvenile F1 male offspring (26-36 day old rat pups), whose parents were exposed at least 71 days prior to their conception, and who themselves were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS male rat pups are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (8)	Group 2 Low-Dose (7)	Group 3 Mid-Dose (8)	Group 4 High-Dose (6)
TP (5.7 – 8.9 g/dL)	4.8 ± 0.4	4.9 ± 0.3	4.8 ± 0.2	5.1 ± 0.1
ALB (3.3 – 6.7 g/dL)	2.8 ± 0.3	2.8 ± 0.2	2.8 ± 0.1	2.9 ± 0.1
ALKP (90 – 205 U/L)	409 ± 43	425 ± 64	411 ± 70	314 ± 57
ALT (23 – 186 U/L)	58 ± 5 (7)	54 ± 7	51 ± 6	51 ± 3
AST (78 – 226 U/L)	93 ± 9	96 ± 11	99 ± 18	81 ± 19
BUN (10 – 25 mg/dL)	12 ± 1	10 ± 3	9 ± 2	**8$^{\Psi}$ ± 2**
CHOL (47 – 92 mg/dL)	75 ± 12	74 ± 16	67 ± 14	71 ± 16
CK (117 – 531 U/L)	241 ± 80	262 ± 95	238 ± 59	264 ± 222
CREA (0.50 – 0.90 mg/dL)	0.24 ± 0.05	0.21 ± 0.04	0.21 ± 0.04	0.24 ± 0.05
GLU (81 – 185 mg/dL)	186 ± 15	166 ± 13	178 ± 15	**160^{Ŧ} ± 18**
TBIL (0.10 – 1.00 mg/dL)	0.10 ± 0.0	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
TRIG (30 – 205 mg/dL)	46 ± 6	37 ± 7	36 ± 10	**29^{Ŧ} ± 5**
Na+ (140 – 156 mEq/L)	147 ± 2	146 ± 1 (6)	148 ± 1	147 ± 2
K+ (4.1 – 6.9 mEq/L)	6.8 ± 0.4	7.0 ± 0.2	7.1 ± 0.6	6.5 ± 0.5
Cl- (95 – 111 mEq/L)	106 ± 1	106 ± 1	107 ± 1	104 ± 3

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 43: Serum chemistries (±SD) measured in adult F1 female offspring (94-105 day old females not selected for breeding), whose parents were exposed at least 71 days prior to their conception, and who were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS adult female rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (8)	Group 2 Low-Dose (6)	Group 3 Mid-Dose (7)	Group 4 High-Dose (7)
TP (5.7 – 8.9 g/dL)	6.2 ± 0.2	**6.9₸ ± 0.2**	6.7 ± 0.3	6.4 ± 0.6
ALB (3.3 – 6.7 g/dL)	3.6 ± 0.2	4.1 ± 0.3	3.7 ± 0.3	3.6 ± 0.5
ALKP (90 – 205 U/L)	99 ± 17	83 ± 16	91 ± 20	90 ± 20 (6)
ALT (23 – 186 U/L)	42 ± 7	49 ± 14	51 ± 16	80 ± 61
AST (78 – 226 U/L)	84 ± 20	88 ± 18	103 ± 15	96 ± 51 (6)
BUN (10 – 25 mg/dL)	15 ± 4	12 ± 3	13 ± 3	14 ± 4
CHOL (47 – 92 mg/dL)	65 ± 14 (7)	78 ± 23	67 ± 13	71 ± 29
CK (117 – 531 U/L)	175 ± 67	197 ± 105	190 ± 66 (6)	157 ± 58
CREA (0.50 – 0.90 mg/dL)	0.45 ± 0.11	0.38 ± 0.04	0.43 ± 0.08	0.40 ± 0.06
GLU (81 – 185 mg/dL)	163 ± 12 (7)	147 ± 25	154 ± 11	164 ± 33
TBIL (0.10 – 1.00 mg/dL)	0.23 ± 0.12	0.30 ± 0.09	0.30 ± 0.08	0.30 ± 0.12
TRIG (30 – 205 mg/dL)	48 ± 11 (7)	62 ± 20	41 ± 9 (6)	39 ± 12 (6)
Na+ (140 – 156 mEq/L)	151 ± 3	152 ± 2	153 ± 2	152 ± 2
K+ (4.1 – 6.9 mEq/L)	7.1 ± 1.4	6.7 ± 0.9	6.4 ± 0.3 (6)	7.1 ± 0.4 (6)
Cl- (95 – 111 mEq/L)	103 ± 1 (7)	104 ± 2	102 ± 2	105 ± 2

₸ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Table 44: Serum chemistries (±SD) measured in adult F1 male offspring (94-105 day old males not selected for breeding), whose parents were exposed at least 71 days prior to their conception, and who were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS adult male rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (7)	Group 2 Low-Dose (7)	Group 3 Mid-Dose (7)	Group 4 High-Dose (6)
TP (5.7 – 8.9 g/dL)	6.2 ± 0.2	6.4 ± 0.5	6.2 ± 0.3	6.3 ± 0.3
ALB (3.3 – 6.7 g/dL)	3.4 ± 0.2	3.5 ± 0.2	3.3 ± 0.2	3.4 ± 0.2
ALKP (90 – 205 U/L)	131 ± 46	145 ± 2	179 ± 28	136 ± 39 (5)
ALT (23 – 186 U/L)	50 ± 9	44 ± 5	49 ± 8	48 ± 6
AST (78 – 226 U/L)	81 ± 20	73 ± 10	90 ± 10	81 ± 6
BUN (10 – 25 mg/dL)	15 ± 3	18 ± 2	16 ± 3	15 ± 3
CHOL (47 – 92 mg/dL)	62 ± 17 (6)	61 ± 19	45 ± 10	57 ± 12
CK (117 – 531 U/L)	141 ± 51	**83$^{\Psi}$ ± 12** (6)	159 ± 53	122 ± 33
CREA (0.50 – 0.90 mg/dL)	0.46 ± 0.10	0.44 ± 0.05	0.41 ± 0.07	0.45 ± 0.05
GLU (81 – 185 mg/dL)	163 ± 22	175 ± 16	159 ± 18	175 ± 22
TBIL (0.10 – 1.00 mg/dL)	0.26 ± 0.08	0.20 ± 0.08	0.21 ± 0.07	0.17 ± 0.08
TRIG (30 – 205 mg/dL)	120 ± 20 (6)	88 ± 9 (6)	**73$^{\text{Ŧ}}$ ± 17**	**83$^{\text{Ŧ}}$ ± 31**
Na+ (140 – 156 mEq/L)	154 ± 2	155 ± 2	152 ± 1	153 ± 3
K+ (4.1 – 6.9 mEq/L)	6.6 ± 0.4	6.6 ± 0.7	6.3 ± 0.8	6.2 ± 0.8
Cl- (95 – 111 mEq/L)	102 ± 2	102 ± 3	102 ± 2	103 ± 2

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 45: Serum chemistries (±SD) measured in adult F1 female offspring (114-125 day old dams), whose parents were exposed at least 71 days prior to their conception, and who were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS adult female rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (7)	Group 2 Low-Dose (7)	Group 3 Mid-Dose (8)	Group 4 High-Dose (7)
TP (5.7 – 8.9 g/dL)	6.9 ± 0.4	7.5 ± 0.2	7.7 ± 0.8	7.3 ± 0.7
ALB (3.3 – 6.7 g/dL)	3.8 ± 0.3	3.9 ± 0.3	4.0 ± 0.5	4.0 ± 0.4
ALKP (90 – 205 U/L)	86 ± 22	96 ± 31	**133Ŧ ± 38**	105 ± 29
ALT (23 – 186 U/L)	49 ± 12	64 ± 14	54 ± 5	62 ± 10
AST (78 – 226 U/L)	73 ± 12	97 ± 29	65 ± 10	81 ± 6
BUN (10 – 25 mg/dL)	14 ± 2	**27Ψ ± 3**	**22Ψ ± 5**	**28Ψ ± 5**
CHOL (47 – 92 mg/dL)	90 ± 11	101 ± 7	94 ± 29	94 ± 17
CK (117 – 531 U/L)	128 ± 59	130 ± 58	116 ± 22	101 ± 28 (6)
CREA (0.50 – 0.90 mg/dL)	0.39 ± 0.07	0.44 ± 0.10	**0.58Ψ ± 0.17**	**0.56Ψ ± 0.08**
GLU (81 – 185 mg/dL)	185 ± 43	187 ± 37	204 ± 23	193 ± 20 (6)
TBIL (0.10 – 1.00 mg/dL)	0.31 ± 0.09	0.44 ± 0.11	0.40 ± 0.12	0.26 ± 0.10
TRIG (30 – 205 mg/dL)	61 ± 19	75 ± 54	86 ± 19	55 ± 13
Na+ (140 – 156 mEq/L)	148 ± 4	**166Ŧ ± 13**	158 ± 8	162 ± 15
K+ (4.1 – 6.9 mEq/L)	6.4 ± 1.2	6.6 ± 1.1	6.2 ± 0.8	7.2 ± 0.7
Cl- (95 – 111 mEq/L)	105 ± 2	102 ± 50	108 ± 5	109 ± 8

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 46: Serum chemistries (±SD) measured in adult F1 male offspring (114-125 day old sires), whose parents were exposed at least 71 days prior to their conception, and who were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS adult male rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (7)	Group 2 Low-Dose (6)	Group 3 Mid-Dose (7)	Group 4 High-Dose (6)
TP (5.7 – 8.9 g/dL)	6.5 ± 0.3	**7.2Ŧ ± 0.5**	6.6 ± 0.2	**7.4Ŧ ± 0.4**
ALB (3.3 – 6.7 g/dL)	3.4 ± 0.2	3.6 ± 0.2	3.3 ± 0.2	3.6 ± 0.2
ALKP (90 – 205 U/L)	139 ± 45	143 ± 24	142 ± 41	160 ± 27
ALT (23 – 186 U/L)	51 ± 4	57 ± 10	56 ± 12	56 ± 9
AST (78 – 226 U/L)	83 ± 10	89 ± 7	82 ± 19	102 ± 25
BUN (10 – 25 mg/dL)	12 ± 1	**19Ŧ ± 2**	15 ± 2	**16Ŧ ± 3**
CHOL (47 – 92 mg/dL)	68 ± 15 (6)	84 ± 14	70 ± 15	89 ± 15
CK (117 – 531 U/L)	101 ± 19 (6)	138 ± 34 (5)	94 ± 27	116 ± 28
CREA (0.50 – 0.90 mg/dL)	0.43 ± 0.05	0.52 ± 0.08	0.41 ± 0.07	0.53 ± 0.12
GLU (81 – 185 mg/dL)	174 ± 11	**205Ŧ ± 21**	190 ± 15	**212Ŧ ± 27**
TBIL (0.10 – 1.00 mg/dL)	0.26 ± 0.08	0.37 ± 0.15	0.26 ± 0.10	0.32 ± 0.15
TRIG (30 – 205 mg/dL)	91 ± 34	115 ± 31	132 ± 51	118 ± 43
Na+ (140 – 156 mEq/L)	153 ± 1	158 ± 9	154 ± 4	166 ± 11
K+ (4.1 – 6.9 mEq/L)	5.9 ± 0.4	6.4 ± 0.8	6.4 ± 0.8	6.6 ± 1.1
Cl- (95 – 111 mEq/L)	105 ± 2	108 ± 5	105 ± 2	111 ± 7

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Table 47: Serum chemistries (±SD) measured in juvenile F2 female offspring (22-27 day old rat pups), whose parents were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS female rat pups are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (7)	Group 2 Low-Dose (5)	Group 3 Mid-Dose (5)	Group 4 High-Dose (6)
TP (5.7 – 8.9 g/dL)	5.2 ± 0.6	4.8 ± 0.1	4.8 ± 0.2	4.9 ± 0.4
ALB (3.3 – 6.7 g/dL)	3.0 ± 0.4	2.4 ± 0.4	2.5 ± 0.2	2.8 ± 0.3
ALKP (90 – 205 U/L)	358 ± 44	**418$^{\Psi}$ ± 9**	**435$^{\Psi}$ ± 53**	420 ± 88
ALT (23 – 186 U/L)	56 ± 7	46 ± 6	53 ± 6	52 ± 10
AST (78 – 226 U/L)	133 ± 19	105 ± 13	113 ± 21	113 ± 26
BUN (10 – 25 mg/dL)	7 ± 1	9 ± 2	9 ± 2	9 ± 2
CHOL (47 – 92 mg/dL)	104 ± 20	100 ± 14	85 ± 10	87 ± 33
CK (117 – 531 U/L)	256 ± 77	217 ± 49	246 ± 139	218 ± 49
CREA (0.50 – 0.90 mg/dL)	0.23 ± 0.05	0.16 ± 0.09	0.16 ± 0.09	0.25 ± 0.10
GLU (81 – 185 mg/dL)	184 ± 31	163 ± 13	192 ± 22	219 ± 20
TBIL (0.10 – 1.00 mg/dL)	0.11 ± 0.04	0.12 ± 0.04	0.18 ± 0.18	0.10 ± 0.00
TRIG (30 – 205 mg/dL)	42 ± 3	41 ± 9	46 ± 13	40 ± 7
Na+ (140 – 156 mEq/L)	148 ± 14	147 ± 8	150 ± 7	147 ± 6
K+ (4.1 – 6.9 mEq/L)	7.0 ± 0.4	7.0 ± 0.5	7.1 ± 0.6	7.8 ± 0.4
Cl- (95 – 111 mEq/L)	108 ± 7	108 ± 5	108 ± 4	104 ± 6

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 48: Serum chemistries (±SD) measured in juvenile F2 male offspring (22-27 day old rat pups), whose parents were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS male rat pups are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (6)	Group 2 Low-Dose (6)	Group 3 Mid-Dose (7)	Group 4 High-Dose (6)
TP (5.7 – 8.9 g/dL)	4.9 ± 0.2	5.1 ± 0.3	4.9 ± 0.3	**7.4$^{\Psi}$ ± 0.4**
ALB (3.3 – 6.7 g/dL)	2.6 ± 0.1	2.7 ± 0.2	2.4 ± 0.2 (6)	**3.6$^{\Psi}$ ± 0.2**
ALKP (90 – 205 U/L)	445 ± 66	499 ± 87	407 ± 62	460 ± 27
ALT (23 – 186 U/L)	56 ± 9	65 ± 5	51 ± 8	56 ± 9
AST (78 – 226 U/L)	114 ± 6	136 ± 22	98 ± 8 (6)	102 ± 25
BUN (10 – 25 mg/dL)	11 ± 3	13 ± 3	8 ± 3	16 ± 3
CHOL (47 – 92 mg/dL)	97 ± 14	93 ± 14	86 ± 8	79 ± 15
CK (117 – 531 U/L)	227 ± 45	254 ± 70	208 ± 51	216 ± 28
CREA (0.50 – 0.90 mg/dL)	0.25 ± 0.05	0.28 ± 0.12	0.19 ± 0.07	**0.53$^{\Psi}$ ± 0.12**
GLU (81 – 185 mg/dL)	192 ± 10	185 ± 18	202 ± 15	212 ± 27
TBIL (0.10 – 1.00 mg/dL)	0.13 ± 0.08	0.14 ± 0.05 (5)	0.13 ± 0.08	**0.32$^{\Psi}$ ± 0.15**
TRIG (30 – 205 mg/dL)	49 ± 10	49 ± 12 (5)	49 ± 15	**118$^{\text{Ŧ}}$ ± 43**
Na+ (140 – 156 mEq/L)	145 ± 5	154 ± 15	145 ± 4	**166$^{\text{Ŧ}}$ ± 11**
K+ (4.1 – 6.9 mEq/L)	7.0 ± 0.2	7.4 ± 0.7	7.3 ± 0.6	6.6 ± 1.1
Cl- (95 – 111 mEq/L)	107 ± 3	111 ± 8	107 ± 1	111 ± 7

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 49: Serum chemistries (±SD) measured in adult F2 female offspring (86-98 day old rats), whose parents were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS adult female rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (8)	Group 2 Low-Dose (7)	Group 3 Mid-Dose (7)	Group 4 High-Dose (7)
TP (5.7 – 8.9 g/dL)	6.6 ± 0.3	6.6 ± 0.2	6.6 ± 0.3	6.5 ± 0.3
ALB (3.3 – 6.7 g/dL)	3.5 ± 0.3	3.5 ± 0.2	3.6 ± 0.2	3.5 ± 0.3
ALKP (90 – 205 U/L)	102 ± 37	97 ± 16	117 ± 50	101 ± 17
ALT (23 – 186 U/L)	55 ± 8	66 ± 27	69 ± 17	63 ± 14
AST (78 – 226 U/L)	78 ± 9	99 ± 39	103 ± 36	104 ± 20
BUN (10 – 25 mg/dL)	13 ± 1	14 ± 3	14 ± 3	12 ± 2
CHOL (47 – 92 mg/dL)	77 ± 12	82 ± 12	68 ± 14	80 ± 10
CK (117 – 531 U/L)	139 ± 51	265 ± 172	214 ± 85	213 ± 69
CREA (0.50 – 0.90 mg/dL)	0.49 ± 0.06	0.43 ± 0.05	0.46 ± 0.05	0.43 ± 0.08
GLU (81 – 185 mg/dL)	201 ± 29	196 ± 23	200 ± 18	193 ± 18
TBIL (0.10 – 1.00 mg/dL)	0.30 ± 0.08	0.39 ± 0.20	0.31 ± 0.11	0.34 ± 0.10
TRIG (30 – 205 mg/dL)	62 ± 16	63 ± 23	62 ± 12	58 ± 14
Na+ (140 – 156 mEq/L)	149 ± 2	147 ± 1	148 ± 1	143 ± 17
K+ (4.1 – 6.9 mEq/L)	6.8 ± 0.7	6.4 ± 0.6	**6.1$^{\Psi}$ ± 0.3**	6.5 ± 0.4
Cl- (95 – 111 mEq/L)	106 ± 2	106 ± 1	106 ± 2	106 ± 1

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 50: Serum chemistries (±SD) measured in adult F2 male offspring (86-98 day old rats), whose parents were exposed *in utero* to GD 19; n=(x). The standard reference ranges for CD® IGS adult male rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control (8)	Group 2 Low-Dose (8)	Group 3 Mid-Dose (7)	Group 4 High-Dose (7)
TP (5.7 – 8.9 g/dL)	6.5 ± 0.3	6.5 ± 0.5	6.3 ± 0.2	6.4 ± 0.3
ALB (3.3 – 6.7 g/dL)	3.4 ± 0.2	3.3 ± 0.3	3.3 ± 0.2	3.3 ± 0.1
ALKP (90 – 205 U/L)	160 ± 44	166 ± 43	181 ± 49	187 ± 57
ALT (23 – 186 U/L)	57 ± 5	58 ± 8	59 ± 8	60 ± 8
AST (78 – 226 U/L)	80 ± 14	96 ± 43	74 ± 14	83 ± 19
BUN (10 – 25 mg/dL)	16 ± 3.1	16 ± 3	16 ± 1	16 ± 2
CHOL (47 – 92 mg/dL)	80 ± 19	72 ± 14	71 ± 11	77 ± 12
CK (117 – 531 U/L)	143 ± 40	245 ± 144	122 ± 38	155 ± 60
CREA (0.50 – 0.90 mg/dL)	0.54 ± 0.05	0.54 ± 0.07	0.50 ± 0.06	0.51 ± 0.07
GLU (81 – 185 mg/dL)	216 ± 39	199 ± 39	216 ± 27	184 ± 24
TBIL (0.10 – 1.00 mg/dL)	0.39 ± 0.15	0.35 ± 0.08	0.34 ± 0.13	0.34 ± 0.10
TRIG (30 – 205 mg/dL)	85 ± 17	99 ± 42	85 ± 35	85 ± 22
Na+ (140 – 156 mEq/L)	147 ± 4	146 ± 2	147 ± 1	148 ± 2
K+ (4.1 – 6.9 mEq/L)	6.6 ± 0.5	6.7 ± 0.8	6.3 ± 0.5	7.1 ± 0.4
Cl- (95 – 111 mEq/L)	104 ± 2	104 ± 1	105 ± 2	103 ± 1

Table 51: Histopathological lesions identified in adult P1 female rats (195-214 day old dams) euthanized 5-8 weeks following a continuous 90-105 day exposure to GD 19, parturition and weaning.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Adrenal Glands	N=8	N=8	N=8	N=8
Number of animals within normal limits	7	8	8	8
- Adenoma; Benign	1	0	0	0
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	7	8
- Necrosis (Hippocampus)	0 -	0 -	1 (1+)	0 -
Heart	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	7	7	8
- Cardiomyopathy; Progressive	0 -	0 -	1 (1+)	0 -
- Hemangiosarcoma, Malignant	0	1	0	0
Kidneys	N=8	N=8	N=8	N=8
Number of animals within normal limits	5	2	3	2
- Infiltration; Lymphohistiocytic	1 (1+)	0 -	0 -	0 -
- Inflammation; Chronic	0 -	0 -	1 (1+)	1 (1+)
- Nephropathy; Chronic Progressive	0 -	4 (3+/1++)	2 (1+/1++)	2 (2+)
- Cast; Proteinacious	1 (1+)	0 -	0 -	0 -
- Mineralization	1 (1+)	3 (3+)	2 (2+)	5 (5+)
Liver	N=8	N=8	N=8	N=8
Number of animals within normal limits	7	3	5	7
- Infiltration; Lymphohistiocytic	1 (1+)	3 (3+)	3 (3+)	1 (1+)
- Necrosis; Hepatocellular	0 -	1 (1++)	0 -	0 -
- Hyperplasia; Biliary	0 -	1 (1+)	0 -	0 -
Mammary Gland	N=8	N=7	N=8	N=7
Number of animals within normal limits	8	7	7	7
- Ectasia; Duct	0 -	0 -	1 (1++)	0 -
Ovaries and Oviducts	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Pancreas	N=7	N=8	N=8	N=8
Number of animals within normal limits	5	6	8	6
- Inflammation; Chronic; Acinus	1 (1+)	2 (1+/1++)	0 -	2 (1+/1++++)
- Hyperplasia; Islet	1 (1+)	0 -	0 -	0 -
Pituitary Gland	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8

Table 51 is continued on next page

Spleen	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Uterus	N=8	N=8	N=8	N=8
Number of animals within normal limits	7	6	5	6
- Dilation	1 (1+++)	2 (1++/1++++)	3 (1+/2++)	2 (1+/1++++)
Uterine Horns	N=8	N=8	N=8	N=8
Number of animals within normal limits	7	7	8	6
- Dilation	1 (1+++)	1 (1+++)	0 -	2 (1++/1+++)

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Table 52: Histopathological lesions identified in adult P1 male rats (163-172 day old breeders) euthanized 3-5 weeks following a continuous 90 day exposure.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Adrenal Glands	N=8	N=8	N=8	N=8
Number of animals within normal limits	7	8	7	6
- Degeneration; Vacuolar	0 -	0 -	1 (1+)	0 -
- Congestion	0 -	0 -	1 (1++)	0 -
- Vacuolation; Lipid	1 (1++)	0 -	0 -	1 (1++)
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Epididymides	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	7	8	8
- Aspermia	0	1	0	0
Heart	N=8	N=8	N=8	N=8
Number of animals within normal limits	2	4	8	8
- Cardiomyopathy; Progressive	**6ǂ** (5+/1++)	4 (4+)	0 -	0 -
Kidneys	N=8	N=8	N=8	N=8
Number of animals within normal limits	2	3	5	6
- Infiltration; Lymphohistiocytic	1 (1+)	0 -	0 -	0 -
- Inflammation; Chronic	0 -	1 (1+)	0 -	0 -
- Nephropathy; Chronic Progressive	5 (3+/2++)	3 (2+/1++)	3 (2+/1++)	2 (2+)
- Cast; Proteinacious	1 (1+)	0 -	0 -	0 -
- Cyst	0	1	0	0
Liver	N=8	N=8	N=8	N=8
Number of animals within normal limits	4	4	3	4
- Infiltration; Lymphohistiocytic	3 (3+)	3 (3+)	4 (4+)	3 (3+)
- Angiectasis	1 (1++)	0 -	0 -	0 -
- Erythropoiesis; Increased	0 -	0 -	1 (1+)	0 -
- Lipidosis	3 (3+)	3 (3+)	2 (2+)	1 (1+)
Pancreas	N=7	N=8	N=8	N=8
Number of animals within normal limits	5	6	3	5
- Inflammation; Chronic; Acinus	0 -	1 (1++)	3 (1+/2++)	2 (1+/1++)
- Inflammation; Chronic; Islet	1 (1++)	1 (1++)	1 (1+)	1 (1+)
- Hyperplasia; Islet	0 -	0 -	1 (1+)	0 -
- Apoptosis; Increased; Acinar	1 (1++)	0 -	0 -	0 -

Table 52 is continued on next page

Pituitary Gland	N=8	N=8	N=8	N=8
Number of animals within normal limits	7	8	8	8
- Hypertrophy	1 (1++)	0 -	0 -	0 -
Spleen	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Testes	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	7	8	8
- Degeneration; Spermatogenic	0 -	1 (1+++)	0 -	0 -

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Ŧ Pearson Chi-square test indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Fisher's Exact Test.

Table 53: Histopathological lesions identified in juvenile F1 female offspring (26-36 day old pups), whose parents were exposed for at least 70 days prior to mating, and who themselves were exposed *in utero* to GD 19.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Adrenal Glands	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Heart	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Kidneys	N=8	N=8	N=8	N=7
Number of animals within normal limits	6	7	6	5
- Inflammation; Sub-acute	1 (1++)	0 -	0 -	0 -
- Inflammation; Chronic	1 (1+)	1 (1+)	0 -	0 -
- Dilation, Pelvis	0 -	0 -	2 (1++/1+++)	0 -
- Mineralization	0 -	0 -	0 -	2 (2+)
Liver	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Mammary Gland	N=5	N=6	N=7	N=8
Number of animals within normal limits	5	6	7	8
Ovaries and Oviducts	N=7	N=8	N=8	N=8
Number of animals within normal limits	7	8	8	7
- Cyst	0	0	0	1
Pancreas	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Pituitary Gland	N=8	N=8	N=7	N=8
Number of animals within normal limits	8	8	7	8
Spleen	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Uterus and Uterine Horns	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Table 54: Histopathological lesions identified in juvenile F1 male offspring (26-36 day old pups), whose parents were exposed for at least 70 days prior to mating, and who themselves were exposed *in utero* to GD 19.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Adrenal Glands	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Heart	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Epididymides	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Kidneys	N=8	N=8	N=8	N=8
Number of animals within normal limits	5	7	6	4
- Basophilia; Tubular	1 (1+)	0 -	0 -	0 -
- Cast; Proteinacious	0 -	0 -	0 -	1 (1+)
- Inflammation; Chronic	2 (1+/1++)	1 (1++)	2 (1+/1++)	1 (1+)
- Mineralization	0 -	0 -	0 -	1 (1+)
- Cyst	1	0	0	1
Liver	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Pancreas	N=8	N=8	N=8	N=7
Number of animals within normal limits	8	8	8	7
Pituitary Gland	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Spleen	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Testes	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Table 55: Histopathological lesions identified in adult F1 female offspring (94-105 day old female not selected for breeding), who were exposed *in utero* to GD 19, and whose parents were exposed for at least 70 days prior to mating.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Adrenal Glands	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	7	8	7
- Degeneration; Vacuolar	0 -	1 (1+)	0 -	1 (1+)
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Heart	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Kidneys	N=8	N=8	N=8	N=8
Number of animals within normal limits	5	7	8	6
- Nephropathy; Chronic Progressive	0 -	0 -	0 -	1 (1+)
- Inflammation; Chronic	1 (1++)	0 -	0 -	0 -
- Infiltration; Lymphohistiocytic	2 (1+/1++)	1 (1+)	0 -	0 -
- Mineralization	0 -	1 (1+)	0 -	1 (1+)
Liver	N=7	N=8	N=8	N=8
Number of animals within normal limits	5	8	4	7
- Infiltration; Lymphohistiocytic	3 (1+/1++)	0 -	3 (3+)	0 -
- Lipidosis	0 -	0 -	1 (1++)	1 (1+)
Mammary Gland	N=7	N=7	N=8	N=7
Number of animals within normal limits	7	7	8	7
Ovaries and Oviducts	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Pancreas	N=8	N=8	N=7	N=8
Number of animals within normal limits	8	8	7	8
Pituitary Gland	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Spleen	N=7	N=8	N=7	N=8
Number of animals within normal limits	7	8	7	8
Uterus	N=8	N=8	N=7	N=8
Number of animals within normal limits	8	8	6	8
- Dilation	0 -	0 -	1 (1+++)	0 -
Uterine Horns	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	7	4	7
- Dilation	0 -	1 (1+)	4 (2+/1++/1+++)	1 (1+)

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Table 56: Histopathological lesions identified in adult F1 male offspring (94-105 day old males not selected for breeding), who were exposed *in utero* to GD 19, and whose parents were exposed for at least 70 days prior to mating.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Adrenal Glands	N=8	N=8	N=8	N=7
Number of animals within normal limits	8	7	6	7
- Congestion	0 -	0 -	1 (1++)	0 -
- Vacuolation; Lipid	0 -	1 (1+)	1 (1++)	0 -
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=8	N=8	N=7
Number of animals within normal limits	8	8	8	7
Epididymides	N=8	N=8	N=8	N=7
Number of animals within normal limits	7	8	8	7
- Infiltration; Lymphocytic	1 (1+)	0 -	0 -	0 -
Heart	N=8	N=8	N=8	N=7
Number of animals within normal limits	4	5	7	6
- Cardiomyopathy; Progressive	4 (2+/2++)	3 (3+)	1 (1++)	1 (1+)
Kidneys	N=8	N=8	N=8	N=7
Number of animals within normal limits	4	1	6	5
- Infiltration; Lymphohistiocytic	0 -	3 (3+)	0 -	0 -
- Dilation; Pelvis	0 -	1 (1++)	1 (1++)	0 -
- Nephropathy; Chronic Progressive	4 (2+/2++)	3 (3+)	1 (1+)	2 (2+)
Liver	N=8	N=8	N=8	N=7
Number of animals within normal limits	3	6	7	5
- Infiltration; Lymphohistiocytic	**5**Ŧ (5+)	1 (1+)	1 (1+)	1 (1+)
- Lipidosis	0 -	1 (1++)	0 -	1 (1+)
Pancreas	N=8	N=8	N=8	N=7
Number of animals within normal limits	5	5	7	5
- Inflammation; Chronic; Acinus	2 (1+/1++)	2 (2+)	0 -	1 (1+)
- Inflammation; Chronic; Islet	1 (1++)	1 (1++)	1 (1+)	2 (2+)
Pituitary Gland	N=8	N=8	N=8	N=7
Number of animals within normal limits	8	8	8	6
- Hypertrophy; Chromophobe	0 -	0 -	0 -	1 (1+)
Spleen	N=8	N=7	N=8	N=7
Number of animals within normal limits	8	7	8	7
Testes	N=8	N=7	N=7	N=7
Number of animals within normal limits	8	7	7	7

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Ŧ Pearson Chi-square test indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Fisher's Exact Test.

Table 57: Histopathological lesions identified in adult F1 female offspring (114-125 day old dams), who were exposed *in utero* to GD 19, and whose parents were exposed for at least 70 days prior to mating.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Adrenal Glands	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Heart	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Kidneys	N=8	N=8	N=8	N=8
Number of animals within normal limits	4	8	6	5
- Nephropathy; Chronic Progressive	0 -	0 -	0 -	1 (1+)
- Inflammation; Sub-acute	0 -	0 -	0 -	1 (1+)
- Inflammation; Lymphoplasmacytic	0 -	0 -	1 (1+)	0 -
- Infiltration; Lymphohistiocytic	2 (1+/1++)	1 (1+)	0 -	0 -
- Mineralization	2 (1+/1++)	0 -	0 -	0 -
- Dilation; Pelvis	1 (1+++)	0 -	1 (1+++)	0 -
- Cast; Proteinacious	0 -	0 -	1 (1++)	1 (1+)
Liver	N=8	N=8	N=8	N=8
Number of animals within normal limits	4	7	8	8
- Infiltration; Lymphohistiocytic	2 (2+)	2 (2+)	1 (1+)	1 (1+)
- Inflammation; Sub-acute; Periportal	1 (1+)	0 -	0 -	0 -
- Lipidosis	0 -	0 -	1 (1+)	0 -
- Necrosis; Hepatocellular	0 -	0 -	0 -	1 (1+)
Mammary Gland	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	7	8	8
- Involution; Acinus	0 -	1 (1+++)	0 -	0 -
Ovaries and Oviducts	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Pancreas	N=8	N=8	N=8	N=8
Number of animals within normal limits	7	8	8	8
- Inflammation; Lymphocytic; Acinus	1 (1+)	0 -	0 -	0 -
Pituitary Gland	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Spleen	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8

Table 57 is continued on next page

Uterus	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Uterine Horns	N=8	N=8	N=8	N=8
Number of animals within normal limits	6	7	5	7
- Dilation	1 (1+)	1 (1++)	2 (2++)	1 (1+)
- Mineralization	0 -	0 -	1 (1+)	0 -

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Table 58: Histopathological lesions identified in adult F1 male offspring (114-125 day old sires), who were exposed *in utero* to GD 19, and whose parents were exposed for at least 70 days prior to mating.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Adrenal Glands	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Epididymides	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	7
- Spermiation; Decreased	0 -	0 -	0 -	1 (1++)
Heart	N=8	N=8	N=8	N=8
Number of animals within normal limits	6	4	6	2
- Cardiomyopathy; Progressive	2 (1+/1++)	4 (2+/2++)	2 (2+)	**6Ŧ** (6+)
Kidneys	N=8	N=8	N=8	N=8
Number of animals within normal limits	3	3	3	2
- Nephropathy; Chronic Progressive	4 (4+)	5 (4+/1++)	4 (1+/3++)	6 (4+/2++)
- Dilation; Pelvis	1 (1++)	0 -	2 (2++)	0 -
- Mineralization	1 (1++)	0 -	0 -	1 (1+)
Liver	N=8	N=8	N=8	N=8
Number of animals within normal limits	5	7	6	6
- Infiltration; Lymphohistiocytic	2 (2+)	1 (1+)	1 (1+)	1 (1+)
- Inflammation; Sub-acute; Periportal	1 (1+)	0 -	0 -	0 -
- Necrosis; Hepatocellular	0 -	0 -	0 -	1 (1+)
- Lipidosis	0 -	0 -	1 (1+)	0 -
Pancreas	N=7	N=8	N=8	N=7
Number of animals within normal limits	5	4	4	2
- Inflammation; Chronic; Acinus	1 (1+)	2 (1+/1++)	3 (1+/2++)	3 (2+/1+)
- Inflammation; Chronic; Islet	2 (2++)	2 (1+/1++)	3 (2+/1++)	2 (1+/1++)
Pituitary Gland	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Spleen	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Testes	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
- Degeneration; Spermatogenic	0 -	0 -	0 -	1 (1++)

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Ŧ Pearson Chi-square test indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Fisher's Exact Test.

Table 59: Histopathological lesions identified in juvenile F2 female offspring (22-27 day old pups), whose parents were exposed *in utero* to GD 19.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Adrenal Glands	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Heart	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Kidneys	N=8	N=8	N=8	N=8
Number of animals within normal limits	6	7	6	7
- Nephropathy; Chronic Progressive	1 (1+)	0 -	0 -	0 -
- Dilation, Pelvis	1 (1++)	0 -	0 -	1 (1+)
- Cyst	0	1	2	0
Liver	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Mammary Gland	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Ovaries and Oviducts	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Pancreas	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Pituitary Gland	N=8	N=7	N=8	N=6
Number of animals within normal limits	8	7	8	6
Spleen	N=8	N=7	N=8	N=8
Number of animals within normal limits	8	7	8	8
Uterus	N=8	N=8	N=7	N=7
Number of animals within normal limits	8	8	7	7
Uterine Horns	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Table 60: Histopathological lesions identified in juvenile F2 male offspring (22-27 day old pups), whose parents were exposed *in utero* to GD 19.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Adrenal Glands	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Heart	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Epididymides	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Kidneys	N=8	N=8	N=8	N=8
Number of animals within normal limits	4	8	7	4
- Inflammation; Chronic	2 (2+)	0 -	0 -	0 -
- Nephropathy; Chronic Progressive	1 (1+)	0 -	0 -	0 -
- Cyst	1	0	1	4
Liver	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Pancreas	N=8	N=8	N=8	N=7
Number of animals within normal limits	8	8	8	7
Pituitary Gland	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Spleen	N=8	N=8	N=8	N=8
Number of animals within normal limits	7	8	8	8
- Erythropoiesis; Increased	1 (1++)	0 -	0 -	0 -
Testes	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Table 61: Histopathological lesions identified in adult F2 female offspring (86-98 day old rats), whose parents were exposed *in utero* to GD 19.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Adrenal Glands	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Heart	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Kidneys	N=8	N=8	N=8	N=8
Number of animals within normal limits	5	6	4	3
- Fibrosis	0 -	0 -	0 -	1 (1+)
- Dilation; Pelvis	1 (1++)	0 -	1 (1++++)	0 -
- Nephropathy; Chronic Progressive	1 (1+)	1 (1++)	0 -	1 (1+/)
- Nephropathy; Alpha 2u Globulin	2 (2+++)	0 -	0 -	0 -
- Mineralization	2 (1+/1++)	2 (1+/1++)	3 (2+/1++)	3 (3+)
Liver	N=8	N=8	N=8	N=8
Number of animals within normal limits	7	8	7	8
- Infiltration; Lymphohistiocytic	1 (1+)	0 -	0 -	0 -
- Lipidosis	0 -	0 -	1 (1++)	0 -
Mammary Gland	N=8	N=8	N=6	N=8
Number of animals within normal limits	8	8	6	8
Ovaries and Oviducts	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Pancreas	N=8	N=8	N=8	N=8
Number of animals within normal limits	7	7	8	6
- Inflammation; Chronic; Acinus	1 (1+)	1 (1+)	0 -	2 (2+)
Pituitary Gland	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Spleen	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Uterus	N=8	N=8	N=8	N=8
Number of animals within normal limits	4	6	8	7
- Dilation	4 (3++/1+++)	2 (1++/1+++)	0 -	1 (1++)
Uterine Horns	N=8	N=8	N=8	N=8
Number of animals within normal limits	4	6	8	8
- Dilation	**4**Ŧ (3++/1+++)	2 (1++/1+++)	0 -	0 -

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Ŧ Pearson Chi-square test indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Fisher's Exact Test.

Table 62: Histopathological lesions identified in adult F2 male offspring (86-98 day old rats), whose parents were exposed *in utero* to GD 19.

Endpoint	Group 1 Control	Group 2 Low-Dose	Group 3 Mid-Dose	Group 4 High-Dose
Adrenal Glands	N=8	N=7	N=8	N=8
Number of animals within normal limits	8	6	8	8
- Vacuolation; Lipid	0 -	1 (1++)	0 -	0 -
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Epididymides	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Heart	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	7	8	8
- Cardiomyopathy; Progressive	0 -	1 (1+)	0 -	0 -
Kidneys	N=8	N=8	N=8	N=8
Number of animals within normal limits	4	4	4	4
- Mineralization	0 -	0 -	1 (1+)	0 -
- Dilation; Pelvis	0 -	0 -	1 (1+++)	2 (2++)
- Nephropathy; Chronic Progressive	3 (2+/1++)	4 (3+/1++)	2 (2+)	2 (1+/1++)
- Nephropathy; Alpha 2u Globulin	2 (2+++)	0 -	0 -	0 -
Liver	N=8	N=8	N=7	N=8
Number of animals within normal limits	8	6	6	8
- Infiltration; Lymphohistiocytic	0 -	2 (2+)	1 (1+)	0 -
Pancreas	N=8	N=8	N=8	N=8
Number of animals within normal limits	4	7	4	7
- Inflammation; Chronic; Acinus	4 (4++)	0 -	4 (2+/2++)	1 (1+)
- Inflammation; Chronic; Islet	0 -	1 (1++)	0 -	0 -
Pituitary Gland	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	7	8	8
- Cyst	0 -	1 Present	0 -	0 -
Spleen	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Testes	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Table 63: Summary of neurobehavioral test results (±SD) in the P1 generation rats (parents) following a continuous 90-day exposure; n=32.

Subject Group	**Group 1** Control	**Group 2** Low-Dose	**Group 3** Mid-Dose	**Group 4** High-Dose
Motor Activity (total activity time in seconds out of 1800 seconds)				
Male Parent	1339 ± 189	1416 ± 172	1342 ± 236	1302 ± 254
Female Parent	[a]	1363 ± 135	1324 ± 133	1422 ± 172
Water Maze Navigation (percentage of time spent in previous platform quadrant)				
Male Parent	33.7 ± 5.8	30.6 ± 6.1	32.9 ± 8.9	38.3 ± 7.9
Female Parent	[a]	30.4 ± 5.9	28.2 ± 7.7	28.8 ± 8.3
Water Maze Navigation (number of crossings over previous platform location)				
Male Parent	3.1 ± 0.6	3.3 ± 0.8	3.4 ± 0.5	4.1 ± 0.7
Female Parent	[a]	3.0 ± 0.8	2.0 ± 0.5	2.3 ± 0.6
Maternal Retrieval P1 (seconds for dam to retrieve 3 PND 2-3 pups removed from nest)				
Female Parent	127.0 ± 100.2	97.4 ± 85.0	153.9 ± 108.6	85.5 ± 94.0

[a] The female parent control group was inadvertently removed from the study prior to motor activity and water maze testing.

Table 64: Summary of neurobehavioral tests results (±SD) in F1 infant rat pups, whose parent(s) were exposed to a continuous 90-day exposure prior to mating; n=32.

Subject Group	**Group 1** Control	**Group 2** Low-Dose	**Group 3** Mid-Dose	**Group 4** High-Dose
Maternal Retrieval F1 (seconds for dam to retrieve 3 PND 2-3 pups removed from nest)				
Female Parent	65.5 ± 37.3	84.8 ± 49.8	89.5 ± 42.7	76.9 ± 40.0
Righting Reflex (seconds for pup at PND 4-5 to rollover from supine to prone position)				
Male Pups	5.44 ± 3.18	**11.03Ŧ ± 5.41**	5.69 ± 4.16	6.09 ± 2.54
Female Pups	7.43 ± 3.29	15.21 ± 11.74	6.66 ± 5.60	7.31 ± 3.73
Separation Distress (number of ultrasonic distress vocalizations emitted per minute at PND 7-8 after pup separation from dam)				
Male Pups	75.6 ± 54.0	88.2 ± 60.8	72.5 ± 51.8	78.7 ± 52.6
Female Pups	64.3 ± 56.1	76.5 ± 56.1	70.2 ± 48.8	71.7 ± 40.6

Ŧ One-way Analysis of Variance (ANOVA) indicated statistically significant differences between the low-dose group and controls, which were validated by the Holm-Sidak method of multiple comparisons.

Table 65: Summary of neurobehavioral tests results (±SD) in F1 adult rat offspring; n=32.

Subject Group	**Group 1** Control	**Group 2** Low-Dose	**Group 3** Mid-Dose	**Group 4** High-Dose
Motor Activity (total activity time in seconds out of 1800 seconds)				
Male Offspring	1362 ± 223	1369 ± 112	1424 ± 117	1249 ± 161
Female Offspring	1359 ± 276	1246 ± 269	1373 ± 136	1276 ± 153
Water Maze Navigation (percentage of time spent in previous platform quadrant)				
Male Offspring	30.2 ± 8.0	35.2 ± 9.3	32.7 ± 7.1	31.9 ± 3.4
Female Offspring	25.4 ± 8.3	28.1 ± 8.3	30.5 ± 9.8	30.9 ± 9.5
Water Maze Navigation (number of crossings over previous platform location)				
Male Offspring	3.2 ± 1.8	3.3 ± 1.8	3.5 ± 1.5	3.9 ± 1.8
Female Offspring	2.4 ± 1.7	2.4 ± 1.3	3.0 ± 2.9	3.1 ± 2.2

Table 66: Summary of neurobehavioral tests results (±SD) in F2 infant rat pups, whose parent(s) were offspring of parents exposed to a continuous 90-day exposure prior to mating; n=32.

Subject Group	**Group 1** Control	**Group 2** Low-Dose	**Group 3** Mid-Dose	**Group 4** High-Dose
Righting Reflex (seconds for pup at PND 4-5 to rollover from supine to prone position)				
Male Pups	8.25 ± 9.46	6.97 ± 7.05	6.24 ± 5.63	6.97 ± 8.07
Female Pups	9.78 ± 9.48	9.78 ± 8.74	11.51 ± 9.28	9.06 ± 12.04
Separation Distress (number of ultrasonic distress vocalizations emitted per minute at PND 7-8 after pup separation from dam)				
Male Pups	104.3 ± 55.4	74.1 ± 48.2	80.4 ± 49.3	74.5 ± 63.8
Female Pups	**109.5[Ŧ] ± 51.1**	67.6 ± 58.0	66.4 ± 57.2	65.8 ± 53.7

Ŧ One-way Analysis of Variance (ANOVA) indicated statistically significant differences between the low-dose group and controls, which were validated by the Holm-Sidak method of multiple comparisons.

Table 67: Summary of neurobehavioral tests results (±SD) in F2 adult rat offspring; n=32.

Subject Group	**Group 1** Control	**Group 2** Low-Dose	**Group 3** Mid-Dose	**Group 4** High-Dose
Motor Activity (total activity time in seconds out of 1800 seconds)				
Male Offspring	1425 ± 172	1317 ± 306	1381 ± 230	1450 ± 173
Female Offspring	1465 ± 188	1271 ± 178	1469 ± 118	1465 ± 62
Water Maze Navigation (percentage of time spent in previous platform quadrant)				
Male Offspring	37.5 ± 10.3	38.4 ± 7.8	39.8 ± 12.2	47.8 ± 10.7
Female Offspring	33.0 ± 9.8	37.0 ± 6.8	35.8 ± 11.2	40.5 ± 10.5
Water Maze Navigation (number of crossings over previous platform location)				
Male Offspring	2.5 ± 2.1	3.4 ± 1.4	4.3 ± 1.9	3.5 ± 1.2
Female Offspring	3.4 ± 2.5	3.5 ± 2.9	2.9 ± 1.4	3.8 ± 2.2

FIGURES

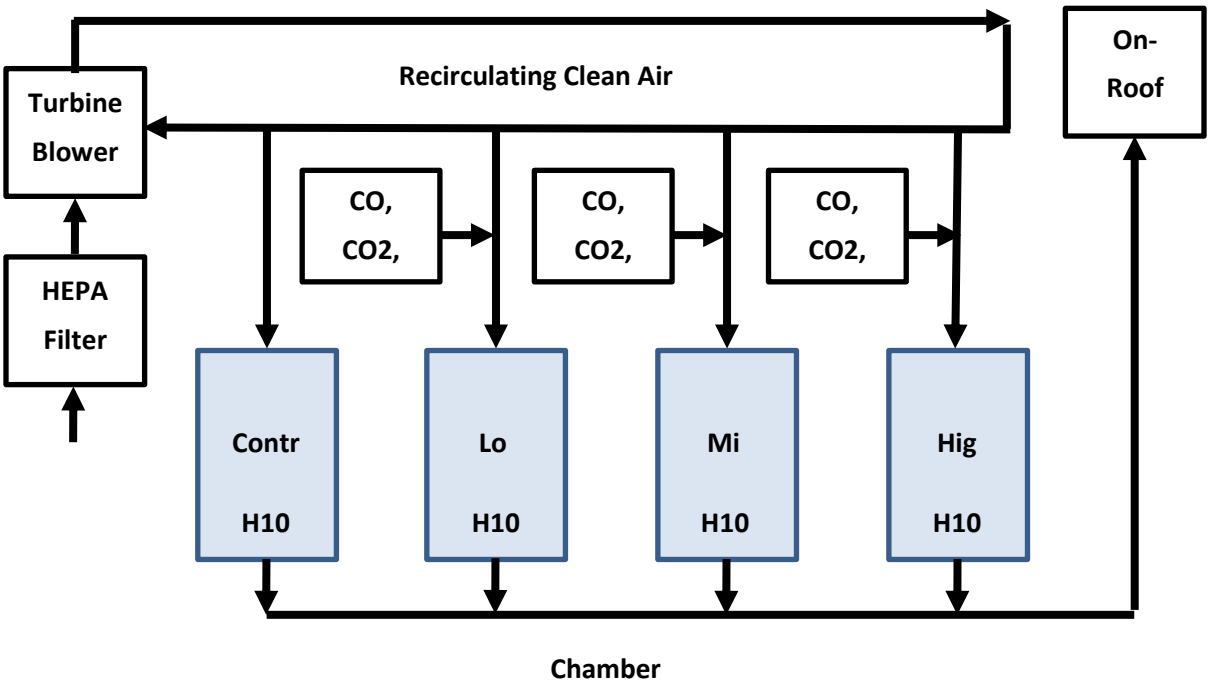

FIGURE 1 Inhalation exposures were conducted in H1000 inhalation chambers for a clean air control and three dose groups. Dilution air for each chamber was directed into the top of each chamber by a turbine blower. The turbine blower recirculated the excess air and pulled the makeup air from the room through a HEPA filter. The test chemicals (CO, CO_2, and N_2) were introduced into the dilution air for each chamber at the required flow rates to achieve the target concentrations for CO, CO_2 and O_2. Nitrogen was used as a test chemical to displace oxygen resulting in reduced oxygen concentrations. All air into the chamber was exhausted from the bottom of the chamber by a roof-mounted blower.

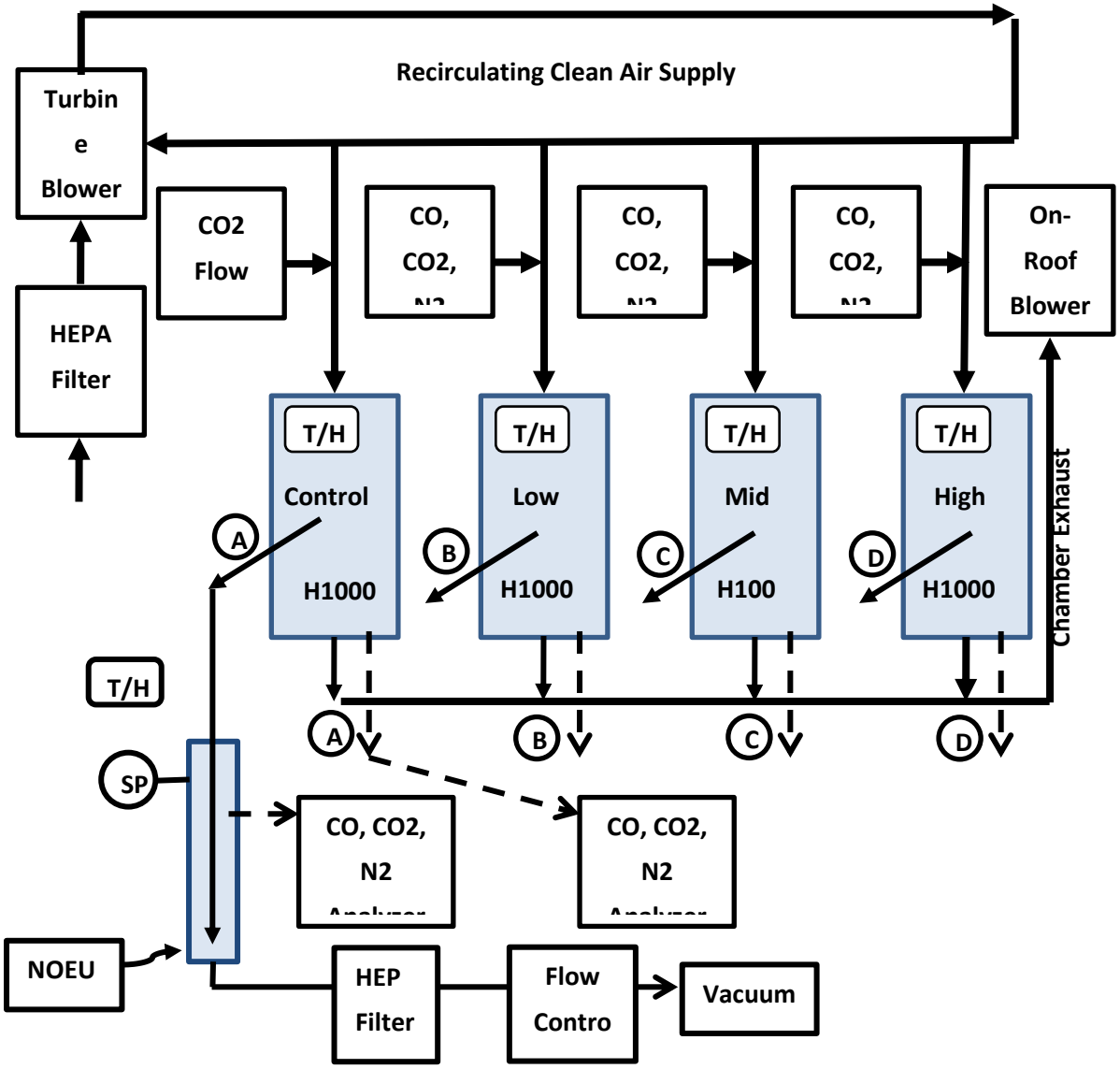

FIGURE 2 Diagrammatic representation of the exposure system: Test atmospheres for inhalation exposures were generated in H1000 inhalation chambers for a clean air control and three dose groups. Dilution air for each chamber was directed into the top of each chamber by a turbine blower. The turbine blower recirculated the excess air and pulled makeup air from the room through a HEPA filter. The test chemicals carbon monoxide (CO), carbon dioxide (CO_2) and nitrogen (N_2) were introduced into the dilution air for each chamber at flow rates to achieve the target concentrations for CO, CO_2 and O_2. Nitrogen was used as a test chemical to displace oxygen resulting in reduced oxygen concentrations. All air into the H1000chamber was exhausted from the bottom of the chamber by a roof-mounted blower. All H1000s were operated under a positive pressure to force the test atmosphere into the inlet of the Nose Only Exposure Unit (NOEU). The NOEU exhaust was set at the target flow rate and the inlet flow was adjusted to maintain a static pressure (SP) near -0.05 inches of water. One analyzer sampled from the NOEU and one analyzer sampled from the H1000. The sample lines from the chambers to the analyzers were rotated (sample points A through D) for the daily exposure chamber of interest. Temperature and humidity (T/H) were monitored in the H1000 inhalation chamber and in the room.

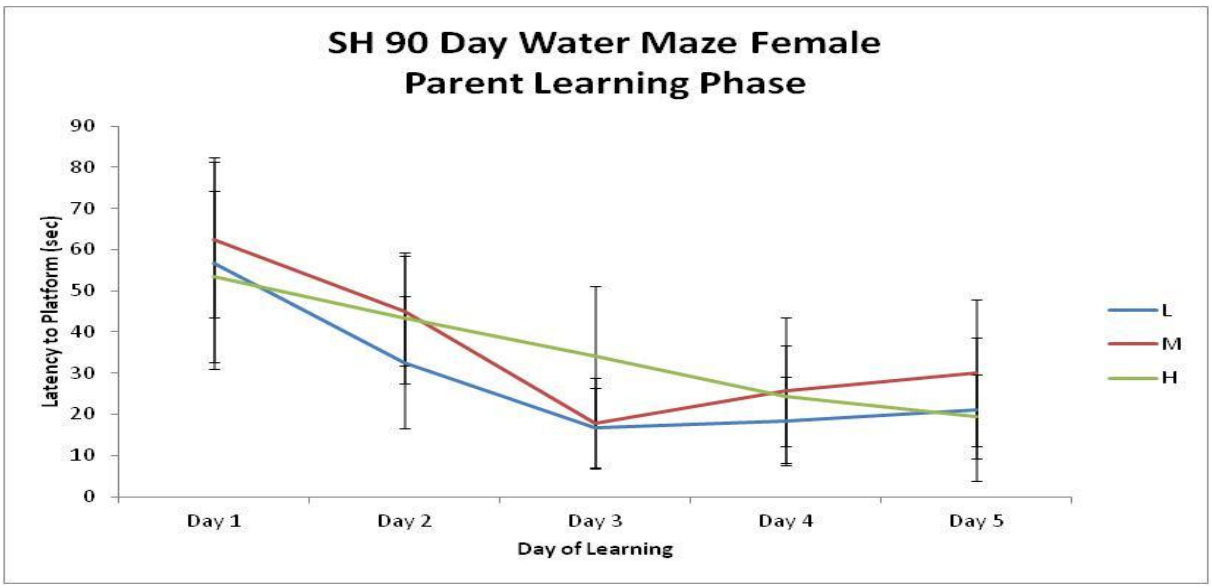

FIGURE 3 The learning performance of the female parents illustrated by the average latency to platform over 5 days given three 90 second trials per day. The 90-day exposure groups included low (L), medium (M), and high (H). Vertical bars represent the standard deviation. On day 3, the high exposure group had a significantly higher latency in comparison to the low and medium exposure groups, (p=0.025).

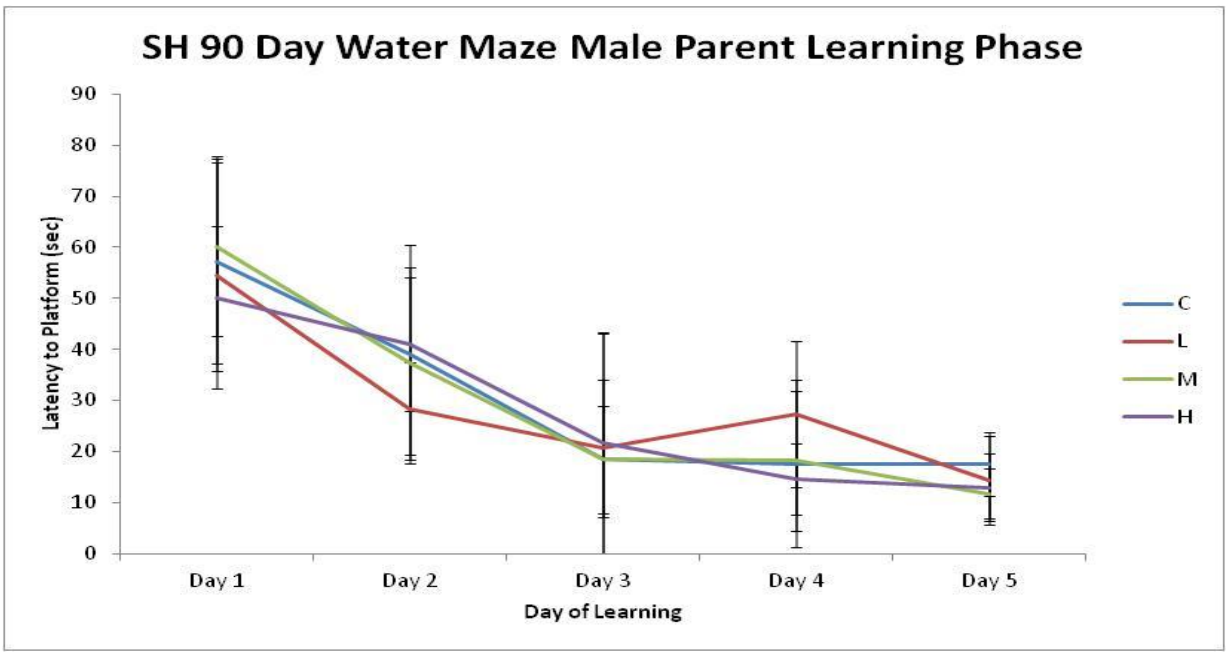

FIGURE 4 The learning performance of the male parents illustrated by the average latency to platform over 5 days given three 90 second trials per day. The 90-day exposure groups included control (C), low (L), medium (M), and high (H). Vertical bars represent the standard deviation. No significant dose-related effects were observed during the 5-day learning phase of water maze navigation.

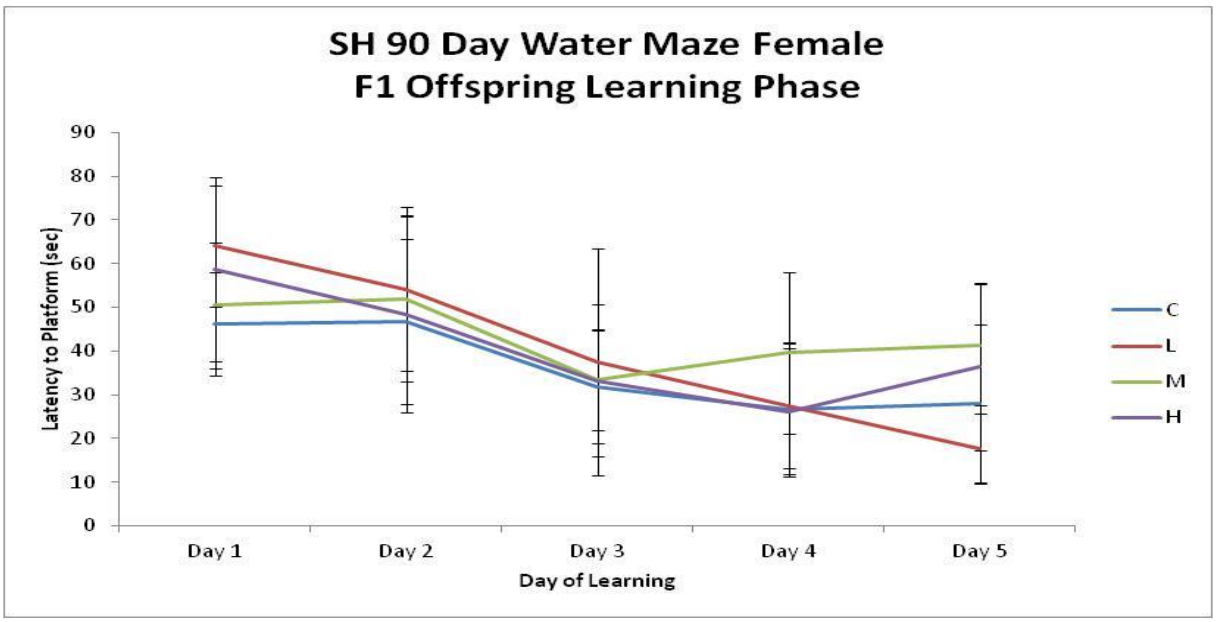

FIGURE 5 The learning performance of the F1 female offspring illustrated by the average latency to find platform over 5 days given three 90 second trials per day. The 90-day exposure groups included control (C), low (L), medium (M), and high (H). Vertical bars represent the standard deviation. On day 5, the medium exposure group had a significantly higher latency in comparison to the low group, (p=0.024).

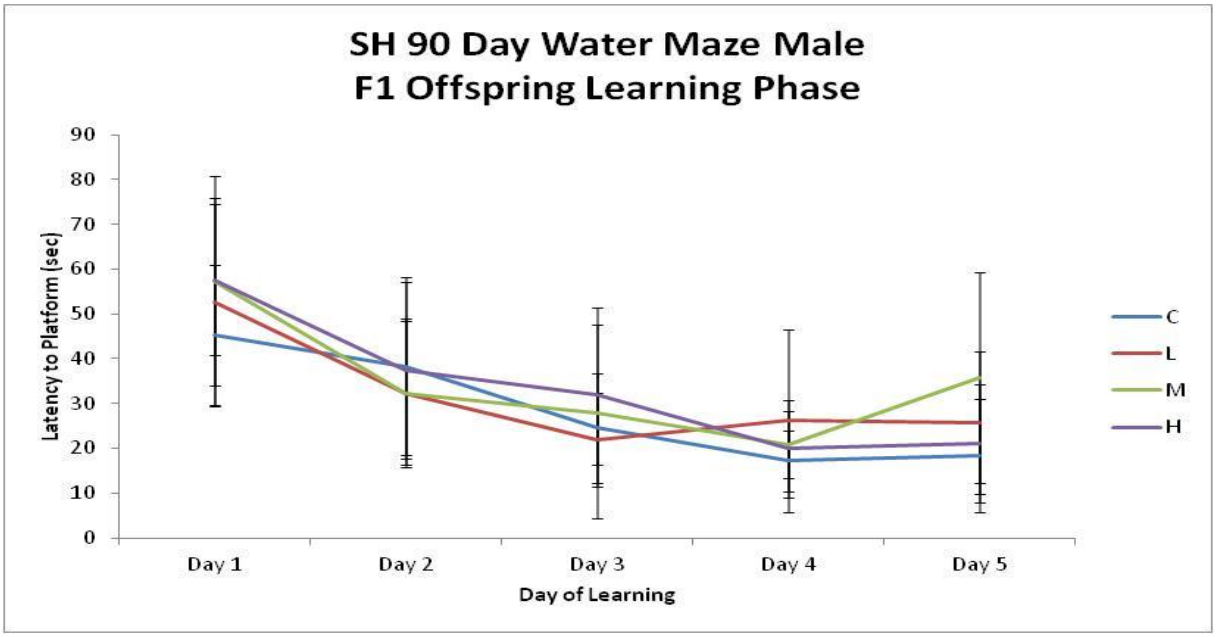

FIGURE 6 The learning performance of the F1 male offspring illustrated by the average latency to platform over 5 days given three 90 second trials per day. The 90-day exposure groups included control (C), low (L), medium (M), and high (H). Vertical bars represent the standard deviation. No significant dose-related effects were observed during the 5-day learning phase of water maze navigation.

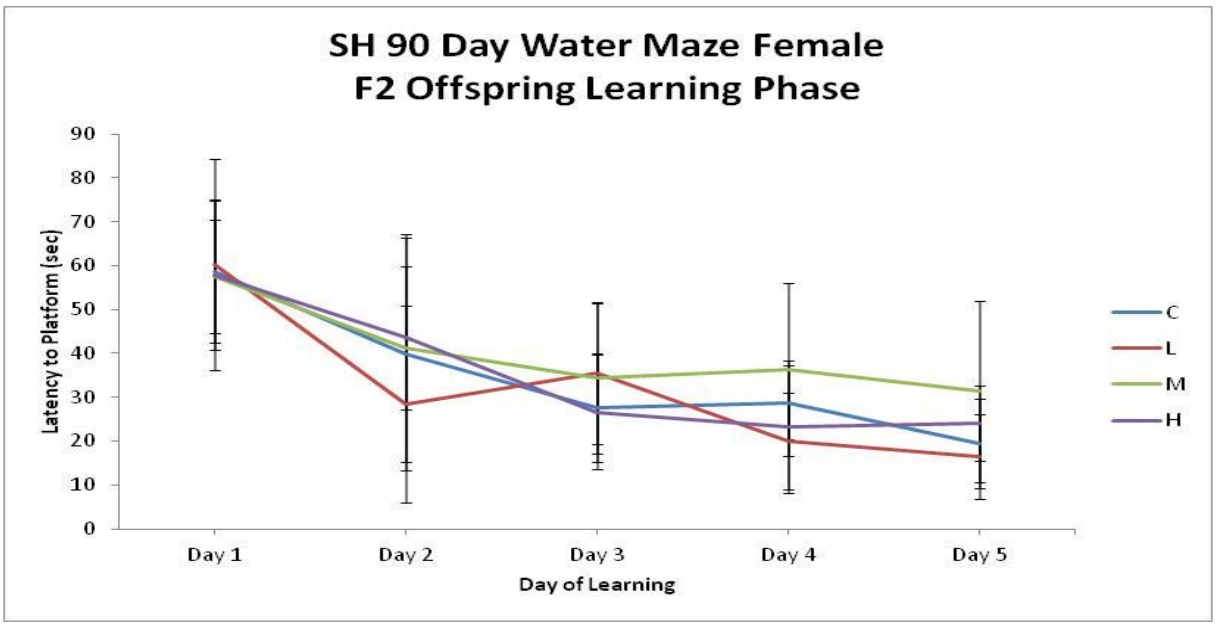

FIGURE 7 The learning performance of the F2 female offspring illustrated by the average latency to find platform over 5 days given three 90 second trials per day. The 90-day exposure groups included control (C), low (L), medium (M), and high (H). Vertical bars represent the standard deviation. No significant dose-related effects were observed during the 5-day learning phase of water maze navigation.

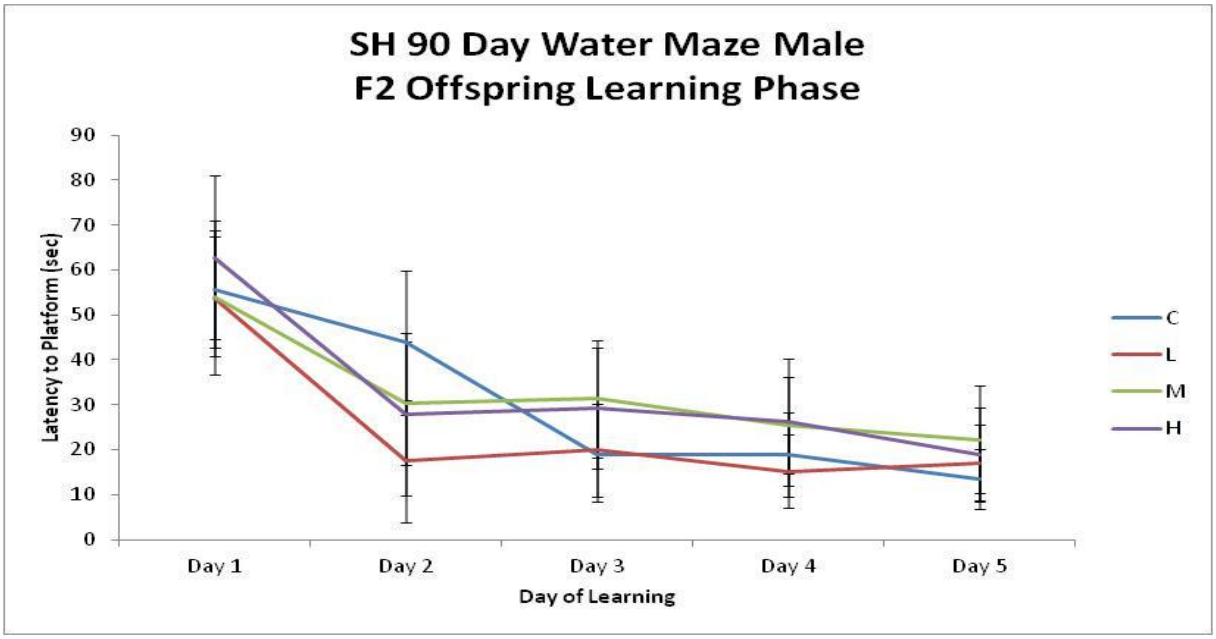

FIGURE 8 The learning performance of the F2 male offspring illustrated by the average latency to find platform over 5 days given three 90 second trials per day. The 90-day exposure groups included control (C), low (L), medium (M), and high (H). Vertical bars represent the standard deviation. On day 2, the low exposure group had a significantly lower latency in comparison to the control group only, (P = 0.018).

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14. ABSTRACT

This study evaluated general, reproductive and developmental effects on male and female rats exposed to mixed atmospheres of three critical submarine air components (CO, CO2, and O2) at concentrations approximating the existing submarine standards for continuous exposure limits (CELs) and emergency exposure limits (24-hour EEL and 1-hour EEL). This report describes a 90-day, two-generation evaluation of the general health and reproductive effects in male and female rats exposed to atmospheres representing the Navy's current limits. This study also evaluated the development and reproductive ability of first generation offspring exposed in utero to gestation day (GD) 19, and the development of the unexposed second generation offspring. Male and Female rats were exposed via whole body inhalation to clean air (, a low-dose gas mixture, a mid-dose gas mixture and a high-dose gas mixture for 23 hours per day for 70 days, followed by a 14-day mating period that was also under exposure. Impregnated dams continued exposure to GD 19. Male and female rats were exposed for 90 to105 days. No adverse reproductive effects were identified in either the exposed parents or first generation offspring during mating, gestation or parturition. There were no adverse changes to the estrous cycle, or in reproductive hormone concentrations, due to the exposures. The only exposure-related effects were reduced weight gains of marginal biological significance and a normal adaptive up-regulation of erythropoiesis, both effects being most notable in male rats from the high-dose group. There were no adverse, dose-related health effects identified in either the exposed parents or offspring based on clinical data (hematology; serum chemistry) or on physiological data (gross pathology; histopathology; organ weights). Additionally, neurobehavioral tests of emotionality, exploratory behavior, motor activity, and cognitive functions (learning and memory) identified no apparent developmental deficits.

15. SUBJECT TERMS
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